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(54) Title: DEAZAAMINOPTERINS FOR TREATMENT OF INFLAMMATION

(57) Abstract

5-Alkyl, 5-alkenyl, 5-alkynyl, and heteroaroyl-5-deazaaminopterins and 5,10-dideazaaminopterins are provided, as well as a method and composition employing such compounds for the treatment of inflammatory disease, such as rheumatoid arthritis.

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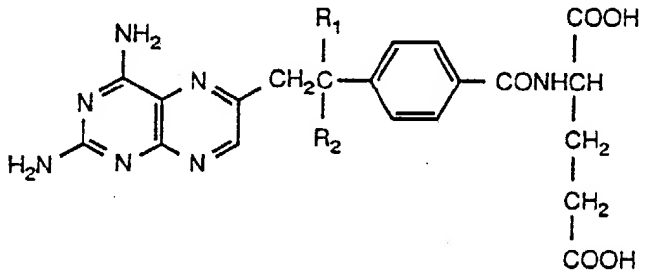
DEAZAAMINOPTERINS FOR TREATMENT OF INFLAMMATION

Field of the Invention

This invention relates to certain deazaaminopterin compounds useful for the treatment of inflammatory disease, such as rheumatoid arthritis, as well as a process for making such compounds and a method of using such compounds.

Background of the Invention

DeGraw et al., U. S. Patent 4,369,319, issued January 19, 1883, disclose a class of 10-deazaaminopterin compounds having the structure of formula:



In the compound 10-deazaaminopterin, R_1 and R_2 are both hydrogen. In the alkyl derivatives of Patent No. 4,369,319, either or both of R_1 and R_2 is alkyl having from one to about eight, preferably one or two, carbon atoms. When only one of R_1 and R_2 is alkyl, the other is hydrogen. Exemplary R_1 and R_2 alkyl include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, iso-amyl, sec-amyl, tert-amyl, hexyl, iso-hexyl, heptyl, iso-heptyl, octyl, iso-octyl, 2-ethyl hexyl and tert-octyl.

DeGraw et al., J.Med.Chem., 17, 552 (1974), report on the synthesis and antifolate activity of 10-deazaaminopterin. The antimicrobial and antitumor activities of the powerful dihydrofolic reductase inhibitors aminopterin and its N-10 methyl derivative, methotrexate, are well known, and numerous analogues have been made to further improve the potency, cell penetration, and toxicity properties of these compounds. As part of a continuing program to investigate structure-activity relationships in folic acid analogues, DeGraw et al. were interested in the effects of replacement of the nitrogen atom in the side chain of aminopterin and reported

on the synthesis and biological activity of 10-deazaaminopterin. Continuing work with 10-deazaaminopterin and its 10-alkyl derivatives led to the discovery of their antileukemic activity, and to their efficacy in treating various ascites tumor systems.

In accordance with U. S. Patent 4,369,319, it was determined that leukemia, as well as other malignancies, including ascitic tumors, can be ameliorated in warm-blooded lower animals by the administration of 10-deazaaminopterin, a nontrivial analogue of methotrexate, the current drug of choice for the treatment of leukemia in the clinic, as well as 10-alkyl derivatives of 10-deazaaminopterin, and it is expected that these compounds will have a similar effect in humans.

Rheumatoid arthritis is an inflammation of the joints arising from infectious, metabolic, or constitutional causes, usually of unknown origin. It can result in serious restriction of movement and even invalidism. Since rheumatoid arthritis is a common disease that affects 2-3 million people in the United State alone, it poses a serious treatment problem. A substantial proportion of affected individuals will develop erosive joint disease and require surgical joint replacement despite therapies including disease-modifying antirheumatic drugs such as gold complexes, penicillamine, antimalarials, and methotrexate. In some patients with intractable rheumatoid arthritis, immunosuppressive agents including azathioprine, methotrexate, cyclophosphamide, and combinations of these drugs have proven beneficial. However, the potential side effects of some of these drugs, including bone marrow toxicity and neoplasia, have limited their frequency of use and the dose that is given.

The disease is one of a number of forms of proliferative disease, and the development of drugs for amelioration or curing the disease has occupied the attention of research organizations for many years, until most recently without appreciable success.

The antifolic acid drug methotrexate has been used as an antitumor agent since 1955. Its cytotoxic action in tumors is related to its ability to inhibit (essentially irreversibly) the key enzyme, dihydrofolate reductase, required for biosynthesis of tetrahydrofolic acid. Tetrahydrofolate is a vital component in one-carbon metabolism in cells, being required for biosynthesis of purine and pyrimidine nucleosides of the DNA and RNA. The drug is a powerful cytotoxic agent whose principal toxicities occur with liver, kidney, and mucosal tissue. Liver toxicity is the paramount concern for use in chronic therapy in a disease such as arthritis.

The ability of methotrexate to affect the inflammatory conditions of rheumatoid arthritis may be linked to its cytotoxic behavior. This may be in the nature of immune suppression and could involve attack on inflammatory phagocytic cells such as macrophages or neutrophils and T-helper cells in the synovial region. Very few methotrexate analogs have been evaluated against arthritis in animals, and there is no clear indication whether the antiarthritic properties are directly proportional to cytotoxicity. Galivan et al., Chem. Biol. Pteridines, DeGuyter, Berlin, 847 (1986), showed that adjuvant arthritis and streptoccocal cell wall arthritis in rats

responded to doses of methotrexate relative to those used in man for treatment of rheumotoid arthritis. They also found that timing of dosage was most important for reduction of inflammation. Both methotrexate and aminopterin were found to inhibit inflammation, but other antifolate compounds that did not possess a 2,4-diaminopyrimidine unit or a benzoylglutamate side chain were ineffective.

Piper et al., J. Med. Chem., 25, 877-880 (1982), prepared the N-10 propyl, octyl, and propargyl analogues of methotrexate for evaluation. Biological evaluations of the three compounds consisted of studies of their effects on enzyme inhibition [(dihydrofolate reductase (EC 1.5.1.3) and thymidylate synthase)], L1210 cell growth inhibition, cellular membrane transport with various murine cell types (L1210, S180, Ehrlich, and epithelial), *in vivo* (mice) activity vs. L1210 leukemia and S180 ascites, and plasma clearance in mice. The *in vivo* results versus S180 ascites offered evidence that the propargyl compound might have a better therapeutic index against this tumor than methotrexate, but no other result from either of these compounds suggested significant superiority over methotrexate.

Taylor et al., J. Org. Chem., 48, 4852-4860 (1983) report that L-5-deazaaminopterin is equipotent with methotrexate both as an inhibitor of bovine liver dihydrofolate reductase and of L1210 murine leukemia cells. It is also equipotent with methotrexate *in vivo* both against L1210 and P388 leukemia in BDF_1 mice.

Piper, J. Med. Chem., 29, 1080-1087 (1986) report evidence indicating that modifications at the 5-and 10-positions of classical folic acid antimetabolites lead to compounds with favorable differential membrane transport in tumor versus normal proliferative tissue. The 5-alkyl-5-deaza analogues were also investigated, including 5-methyl-5-deazaaminopterin, 5-methyl-5-deazamethotrexate, and 5-methyl-10-ethyl-5-deazaaminopterin. Biological evaluation of the 5-methyl-5-deaza analogues, together with previously reported 5-deazaaminopterin and 5-deazamethotrexate, for inhibition of dihydrofolate reductase (DHFR) isolated from L1210 cells and for their effect on cell growth inhibition, transport characteristics, and net accumulation of polyglutamate forms in L1210 cells revealed the analogues to have essentially the same properties as the appropriate parent compound, aminopterin or methotrexate, except that the last two were approximately 10 times more growth inhibitory than methotrexate. In *in vivo* tests against P388/O and P388/methotrexate leukemia in mice, the analogues showed activity comparable to that of methotrexate, with the more potent 20 producing the same response in the P388/O test as methotrexate but at one-fourth the dose; none showed activity against P388/methotrexate.

DeGraw et al., J. Heterocyclic Chem., 88, 1 (9186), describe the synthesis of 5,10-dideazaaminopterin by two independent routes. Condensation of the piperidine enamine of 4-*p*-carbomethoxyphenylbutyraldehyde with ethoxymethylenemalononitrile followed by treatment of the resultant arylethylenaminomalononitrile with methanolic ammonia produced 2-amino-3-

cyano-5-*p*-carbomethoxyphenethylpyridine. Cyclization of the aminocyanopyridine with guanidine afforded 4-amino-4-deoxy-5,10-dideazapteroic acid. Coupling of the pteroate intermediate with glutamate yielded the target 5,10-dideazaaminopterin. Alternatively, reduction of 2,4-diamino-6-formyl-5-deazapteridine with sodium borohydride gave the 6-hydroxymethyl compound. Conversion to the bromide was followed by alkylation of dimethyl homoterephthalate to afford methyl 4-amino-4-deoxy-10-carbomethoxy-5,10-dideazapteroate. Decarboxylation with ester cleavage (sodium cyanide in dimethyl sulfoxide at 180°) also gave the diaminopteroic acid. They reported that 5,10-dideazaaminopterin was an effective growth inhibitor of folate dependent bacteria, *S. faecium* and *L. casei*.

Sirotnak et al, Cancer Research, 5686-5691 (1988), describe studies examining a new class of 4-aminofolate analogues modified by an N to C conversion and alkyl substitution at the N-5 position of aminopterin and methotrexate. All of these analogues were equivalent to aminopterin and methotrexate as inhibitors of tumor cell dihydrofolate reductase (K_I = 3.49-5.16 pM).

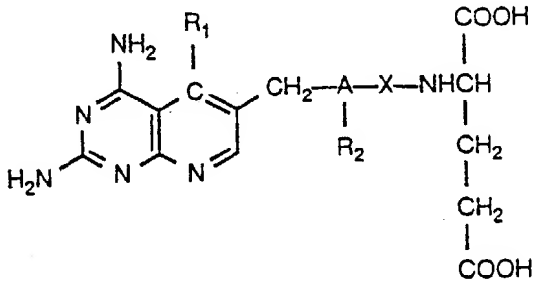
Piper et al., Chemistry and Biology of Pteridines, 1989, Walter de Gruyter & Co., Berlin - New York, state that modifications at the 5- and 10-positions of the classical antifolate structure have produced agents with antitumor activity superior to that of methotrexate. Examples are found in 5-alkyl-5-deaza analogues of aminopterin and methotrexate and in the 10-deazaaminopterin series, particularly 10-ethyl-10-deazaaminopterin whose ongoing clinical trials have produced highly favorable results). The 5-alkyl (methyl or ethyl) derivatives of 5-deazaaminopterin and 5-deazamethotrexate are equivalent to the parent compounds and to aminopterin and methotrexate as inhibitors of tumor cell dihydrofolate reductase, and overall, the activity of the 5-alkyl-5-deazamethotrexate derivatives appears to be equivalent to that of the 10-alkyl-10-deazaaminopterin types. In continuing studies on the effects of changes at positions 5 and 10, they synthesized 10-ethyl-5-methyl-5,10-dideazaaminopterin, 10-propargyl-5-deazaaminopterin, and 10-propargyl-5-methyl-5-deazaaminopterin. The syntheses and available data from biological evaluations are reported.

DeGraw et al., J. Med. Chem., 83, 678 (1990), report the synthesis of the 10-methyl and 10-ethyl analogues of 5,10-dideazatetrahydrofolic acid (DDTHF), a potent inhibitor of glycinamide ribotide (GAR) formyltransferase. Key intermediates in the process were 10-methyl- and 10-ethyl-4-amino-4-deoxy-5,10-dideazapteroic acid. Condensation of the piperidine enamines of branched 4-(*p*-carbomethoxyphenyl)butyraldehydes with (acetoxymethylene)malo-nonitrile afforded 1,1-dicyano-4-piperidinobutadiene. Subsequent reaction with alcoholic ammonium hydroxide yielded the appropriately substituted 2-amino-3-cyanopyridines. Ring closure with guanidine gave 10-methyl- and 10-ethyl-4-amino-4-deoxy-5,10-dideazapteroic acids. Coupling with diethyl glutamate followed by ester hydrolysis afforded 10-alkyl-5,10-dideazaaminopterin analogues which were effective inhibitors of DHFR derived from L1210, but were less potent than methotrexate for inhibition of growth of L1210 in culture.

What is needed is an effective treatment for inflammatory disease, such as rheumatoid arthritis, which exhibits relatively low toxicity compared to current treatments.

Description of the Invention

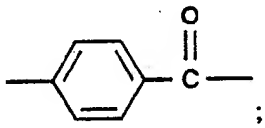
In accordance with the present invention, 5-alkyl, 5-alkenyl, 5-alkynyl, and heteroaroyl-5-deazaaminopterin and 5,10-dideazaaminopterin compounds are provided having the structure of Formula I:



wherein

A is CH or N;

X is one of



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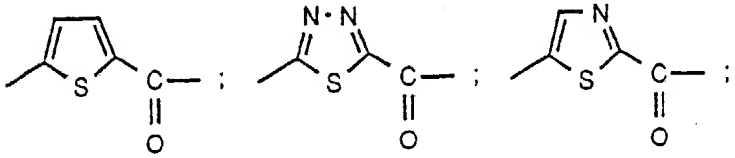
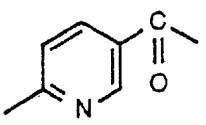
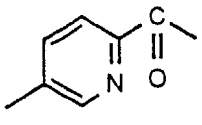




and



and R_1 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

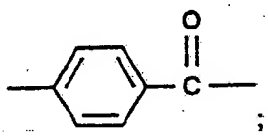
Exemplary R_1 and R_2 alkyl include methyl, ethyl, propyl, iso-propyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, iso-amyl, sec-amyl, tert-amyl, hexyl, iso-hexyl, heptyl, iso-heptyl, octyl, iso-octyl, 2-ethyl hexyl and tert-octyl.

Exemplary R_2 alkenyl include allyl, 1-propenyl, crotyl (2-butenyl), 2-pentenyl, 4-pentenyl, 2-hexenyl, 5-hexenyl, 3-isopropenyl, 3-isobutenyl, and 4-octenyl.

Exemplary R_2 alkynyl include propargyl, 2-butynyl, 3-butynyl, 4-pentynyl, 5-hexynyl, and 7-octynyl.

The invention also provides a process of treating rheumatoid arthritis and other proliferative diseases, which comprises administering to a warm-blooded animal having an inflammation of the joints or other evidence of the disease, a therapeutic nontoxic amount of a 5-alkyl, 5-alkenyl, 5-alkynyl, or heteroaroyl-5-deazaaminopterin or 5,10-dideazaaminopterin compound as defined by Formula I hereinabove, as such or in the form of a pharmaceutically acceptable salt thereof. These salts are formed with one or more free NH_2 groups and/or COOH groups of the 5-deazaaminopterin or 5,10-dideazaaminopterin compound.

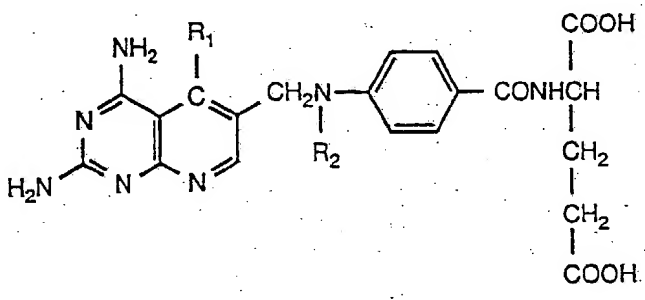
Those compounds in which A is N, X is



;

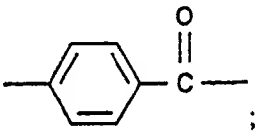
and R_1 and R_2 have from three to about eight carbon atoms, preferably from three to about five carbon atoms, are believed to be novel, and in addition, are exceptionally effective in the treatment of arthritis, in fact from six to ten times more effective than those compounds in which R_1 and R_2 have one or two carbon atoms, so as to constitute a delineated subclass within the larger genus. These compounds are therefore especially preferred.

This subclass of compounds within the invention accordingly is defined by the structure of Formula II:



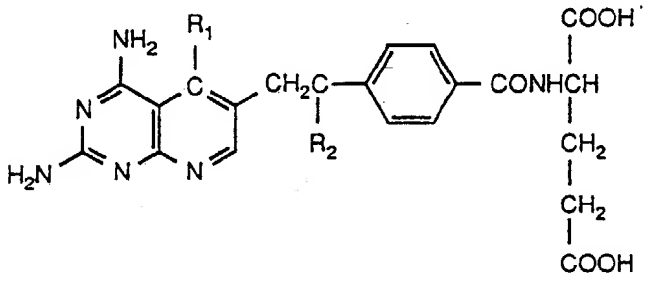
wherein R_1 and R_2 are alkyl, alkenyl, or alkynyl having from three to about eight, preferably from three to five, carbon atoms.

Those compounds in which A is CH and X is



and R_1 and R_2 are alkyl, alkenyl, or alkynyl having from three to about eight carbon atoms, preferably from three to about five carbon atoms, also are believed to be novel, and should be effective in the treatment of arthritis.

This subclass of compounds within the invention accordingly is defined by the structure of Formula III:



wherein R_1 and R_2 are alkyl, alkenyl, or alkynyl having from three to about eight, preferably from three to five, carbon atoms.

Exemplary 5-deazaaminopterin and 5,10-dideazaaminopterin compounds falling within Formulae I, II, or III are shown in the following Table IA.

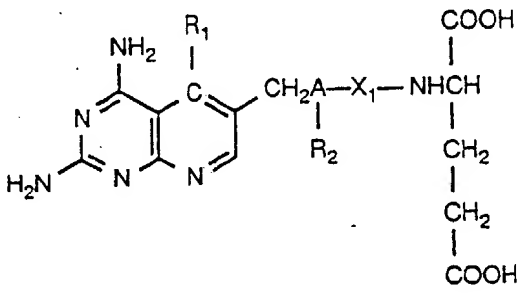
Table IA

Compound No.	R_1	R_2
1	H	H
2	H	CH_3
3	H	C_2H_5
4	H	C_3H_7
5	H	$CH_2=CHCH_2$-
6	H-	$CH\equiv CCH_2$-
7	H	C_5H_{11}
8	H	C_8H_{17}
9	CH_3	H
10	CH3	CH_3
11	CH_3	C_2H_5
12	CH_3	C_3H_7
13	CH_3	$CH_2=CHCH_3$
14	CH_3	$CH\equiv CCH_2$
15	CH_3	C_8H_{17}
16	C_2H_5	H
17	C_2H_5	CH_3
18	C_2H_5	C_2H_5
19	C_2H_5	$CH_2=CHCH_2$

Table IA

Compound No.	R_1	R_2
20	C_2H_5	$CH{\equiv}CCH_2$
21	C_3H_7	H
22	C_3H_7	CH_3
23	*i*-C_3H_7	H
24	*i*-C_3H_7	CH_3
25	n-C_4H_9	H
26	n-C_4H_9	CH_3
27	CH_2=CH-CH_2-	H
28	CH_2=$CHCH_2$	CH_3
29	$CH{\equiv}CCH_2$	H
30	$CH{\equiv}CCH_2$	CH_3
31	C_5H_{11}	H
32	C_8H_{17}	H

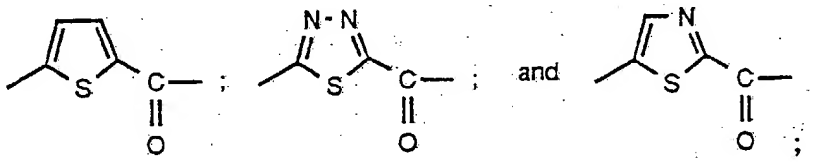
One subclass of thienyl compounds and thienyl analogues within the invention is defined by Formula IV:



wherein

A is N or CH;

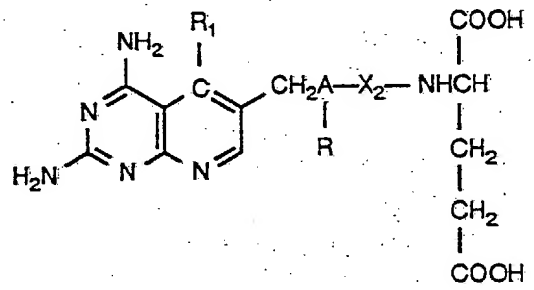
X_1 is one of



and R_1 is hydrogen or alkyl having from one to about eight, preferably from one to three, carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight, preferably from one to three, carbon atoms.

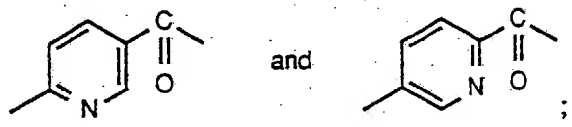
Another subclass of pyridyl compounds within the invention is defined by Formula V:



wherein

A is CH or N;

X_2 is one of



and R_1 is hydrogen or alkyl having from one to about eight, preferably from one to three, carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight, preferably from one to three, carbon atoms.

Exemplary deazaaminopterin compounds falling within Formulae I, IV, and V are shown in the following Tables IB and IIB.

Table IB

5-Deazaaminopterin Compounds (A = N)

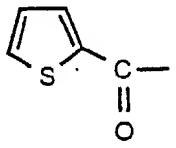
Compound No.	R_1	R_2	X
Example A'			
1	H	H	
2	H	CH_3	
3	H	C_2H_5	
4	H	C_3H_7	
5	H	i-C_3H_7	
6	H	C_4H_9	
7	H	C_5H_{11}	
8	H	C_8H_{17}	
9	H	CH_2=$CHCH_2$-	
10	H	HC≡CCH_2-	
Example B'			
11	CH_3	H	

Table IB

5-Deazaaminopterin Compounds (A = N)

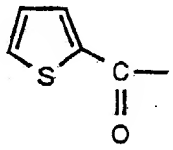
Compound No.	R_1	R_2	X
Example C'			
12	CH_3	CH_3	
13	CH_3	C_2H_5	
14	CH_3	C_3H_7	
15	CH_3	i-C_3H_7	
16	CH_3	CH_2=CH_2CH_2-	
17	CH_3	HC≡CCH_2-	
18	C_2H_5	H	
Example D'			
19	C_2H_5	CH_3	
20	C_2H_5	C_2H_5	
Example E'			
21	C_3H_7	H	
22	C_3H_7	CH_3	

Table IB

5-Deazaaminopterin Compounds (A = N)

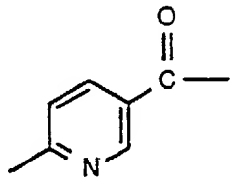
Compound No.	R_1	R_2	X
23	H	H	
24	H	CH_3	
25	H	C_3H_7	
26	H	C_4H_9	
27	H	CH_2=$CHCH_2$-	
28	H	HC≡CCH_2-	
29	CH_3	H	
30	CH_3	C_2H_5	
31	CH_3	C_5H_{11}	
32	C_2H_5	H	
33	C_2H_5	CH_3	
34	C_3H_7	H	

Table IB

5-Deazaaminopterin Compounds (A = N)

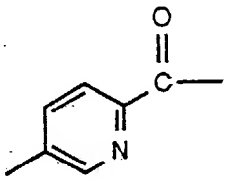
Compound No.	R1	R2	X
35	H	H	
36	H	CH_3	
37	H	C_3H_7	
38	H	C_4H_9	
39	H	CH_2=$CHCH_2$-	
40	H	HC≡$CHCH_2$-	
41	CH_3	H	
42	CH_3	C_2H_5	
43	CH_3	C_5H_{11}	
44	C_2H_5	H	
45	C_2H_5	CH_3	
46	C_3H_7	H	

Table IB

5-Deazaaminopterin Compounds (A = N)

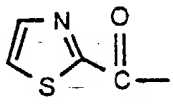
Compound No.	R1	R2	X
47	H	H	
48	H	CH_3	
49	H	C_3H_7	
50	H	C_4H_9	
51	H	CH_2=$CHCH_2$-	
52	H	HC≡$CHCH_2$-	
53	CH_3	H	
54	CH_3	C_2H_5	
55	CH_3	C_5H_{11}	
56	C_2H_5	H	
57	C_2H_5	CH_3	
58	C_3H_7	H	

Table IB

5-Deazaaminopterin Compounds (A = N)

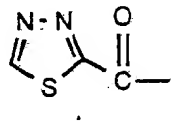
Compound No.	R_1	R_2	X
59	H	H	
60	H	CH_3	
61	H	C_3H_7	
62	H	C_4H_9	
63	H	$CH_2=CHCH_2$-	
64	H	$HC\equiv CHCH_2$-	
65	CH_3	H	
66	CH_3	C_2H_5	
67	CH_3	C_5H_{11}	
68	C_2H_5	H	
69	C_2H_5	CH_3	
70	C_3H_7	H	

Table IIB

5,10-Diazaaminopterin Compounds (A = CH)

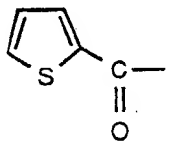
Compound No.	R_1	R_2	X
Example F'			
71	H	H	
72	H	CH_3	
73	H	C_2H_5	
74	H	C_3H_7	
75	H	i-C_3H_7	
76	H	C_4H_9	
77	H	C_5H_{11}	
78	H	C_8H_{17}	
79	H	CH_2=$CHCH_2$-	
80	H	HC≡CCH_2-	
81	CH_3	H	
82	CH_3	CH_3	
83	CH_3	C_2H_5	

Table IIB

5,10-Diazaaminopterin Compounds (A = CH)

Compound No.	R_1	R_2	X
84	CH_3	C_3H_7	
85	CH_3	i-C_3H_7	
86	CH_3	CH_2=CH_2CH_2-	
87	CH_3	HC≡CCH_2-	
88	C_2H_5	H	
89	C_2H_5	CH_3	
90	C_2H_5	C_2H_5	
91	C_3H_7	H	
92	C_3H_7	CH_3	

Table IIB

5,10-Diazaaminopterin Compounds (A = CH)

Compound No.	R_1	R_2	X
Example G'			
93	H	H	
94	H	CH_3	
95	H	C_3H_7	
96	H	C_4H_9	
97	H	CH_2=$CHCH_2$-	
98	H	HC≡$CHCH_2$-	
Example H'			
99	CH_3	H	
100	CH_3	C_2H_5	
101	CH_3	C_5H_{11}	
102	C_2H_5	H	
103	C_2H_5	CH_3	
104	C_3H_7	H	

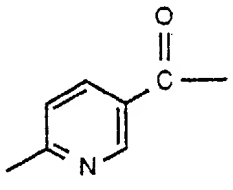


Table IIB

5,10-Diazaaminopterin Compounds (A = CH)

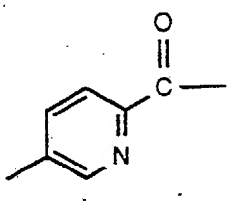
Compound No.	R_1	R_2	X
105	H	H	
106	H	CH_3	
107	H	C_3H_7	
108	H	C_4H_9	
109	H	CH_2=$CHCH_2$-	
110	H	HC≡$CHCH_2$-	
111	CH_3	H	
112	CH_3	C_2H_5	
113	CH_3	C_5H_{11}	
114	C_2H_5	H	
115	C_2H_5	CH_3	
116	C_3H_7	H	

Table IIB

5,10-Diazaaminopterin Compounds (A = CH)

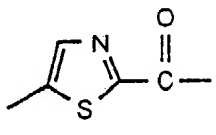
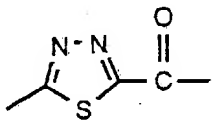
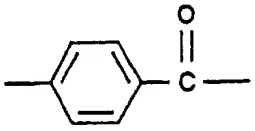
Compound No.	R1	R2	X
117	H	H	
118	H	CH_3	
119	H	C_3H_7	
120	H	C_4H_9	
121	H	CH_2=$CHCH_2$-	
122	H	HC≡$CHCH_2$-	
123	CH_3	H	
124	CH_3	C_2H_5	
125	CH_3	C_5H_{11}	
126	C_2H_5	H	
127	C_2H_5	CH_3	
128	C_3H_7	H	

Table IIB

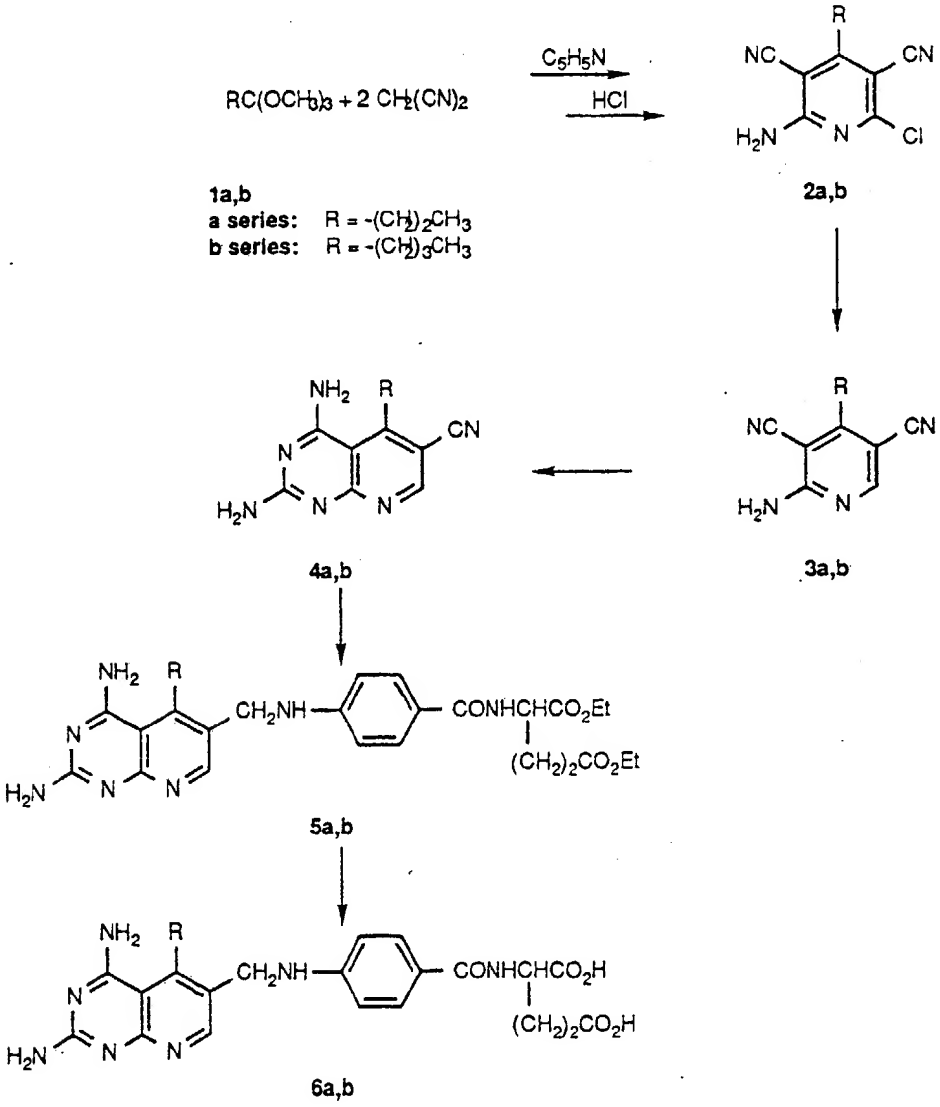
5,10-Diazaaminopterin Compounds (A = CH)

Compound No.	R_1	R_2	X
129	H	H	
130	H	CH_3	
131	H	C_3H_7	
132	H	C_4H_9	
133	H	$CH_2{=}CHCH_2$-	
134	H	$HC{\equiv}CHCH_2$-	
135	CH_3	H	
136	CH_3	C_2H_5	
137	CH_3	C_5H_{11}	
138	C_2H_5	H	
139	C_2H_5	CH_3	
140	C_3H_7	H	

The synthesis of the compounds of Formula II, wherein A is N and X is



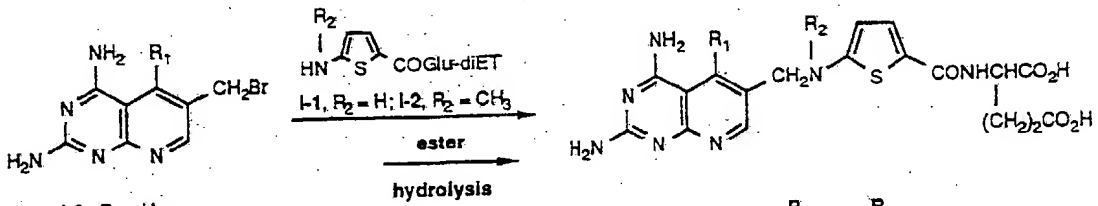
is adapted from that reported by Piper et al., J. Med. Chem., 29, 1080-1087 (1986), as summarized in Procedure I below.



Procedure I

The synthesis of compounds of Formulae I, IV, and V wherein A is N and X is any of the heterocyclic rings set out for these formulae can be carried out by Procedure II, summarized hereinbelow.

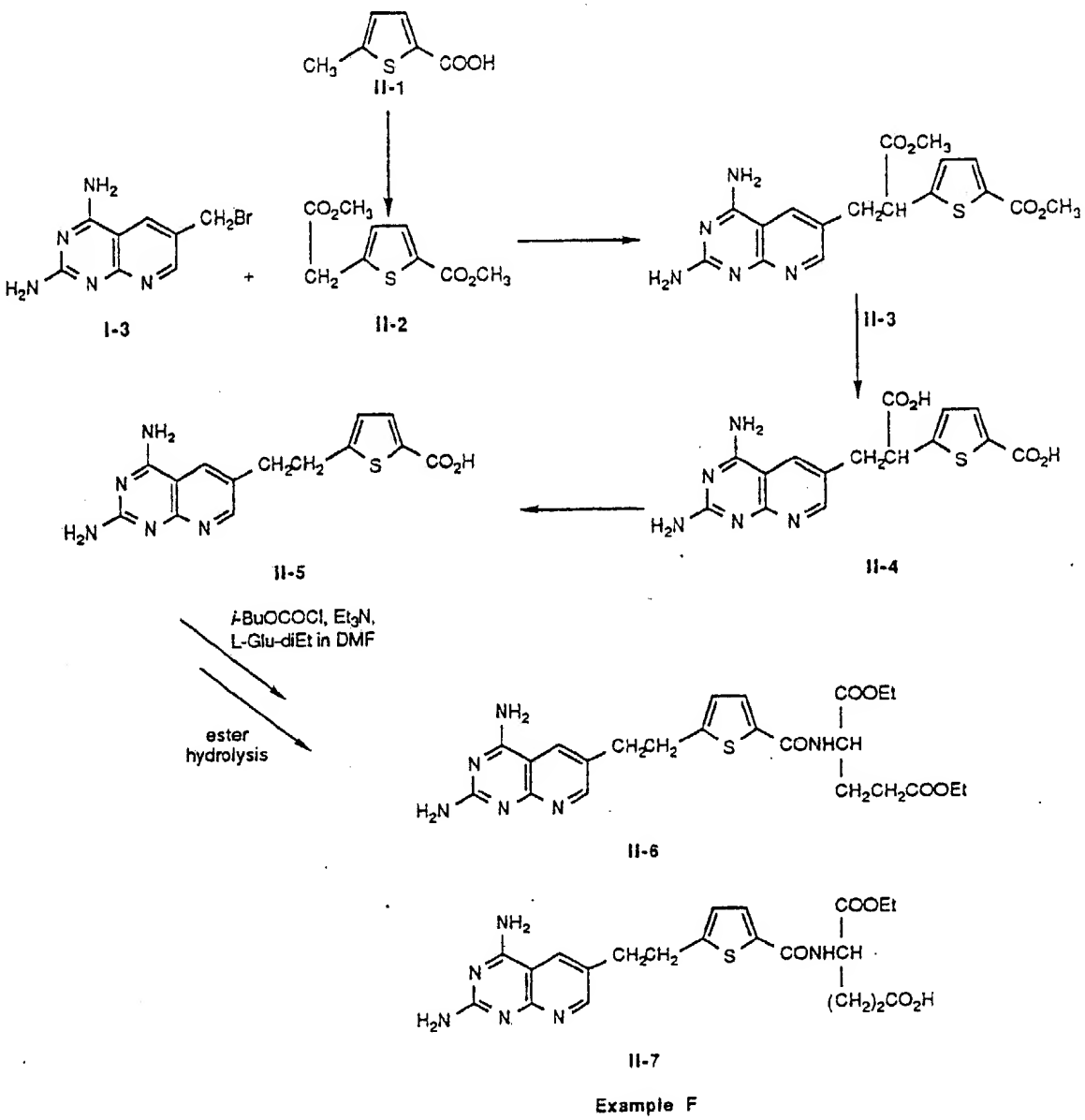
The synthesis of compounds of Formulae I, IV, and V wherein A is CH can be carried out by either of Procedures III and IV, summarized hereinbelow. Procedures III and IV are believed to be novel and are the subject of co-pending patent applications.



I-3: R_1 = H
I-4: R_1 = CH_3
I-5: R_1 = C_2H_5
I-6: R_1 = C_3H_7

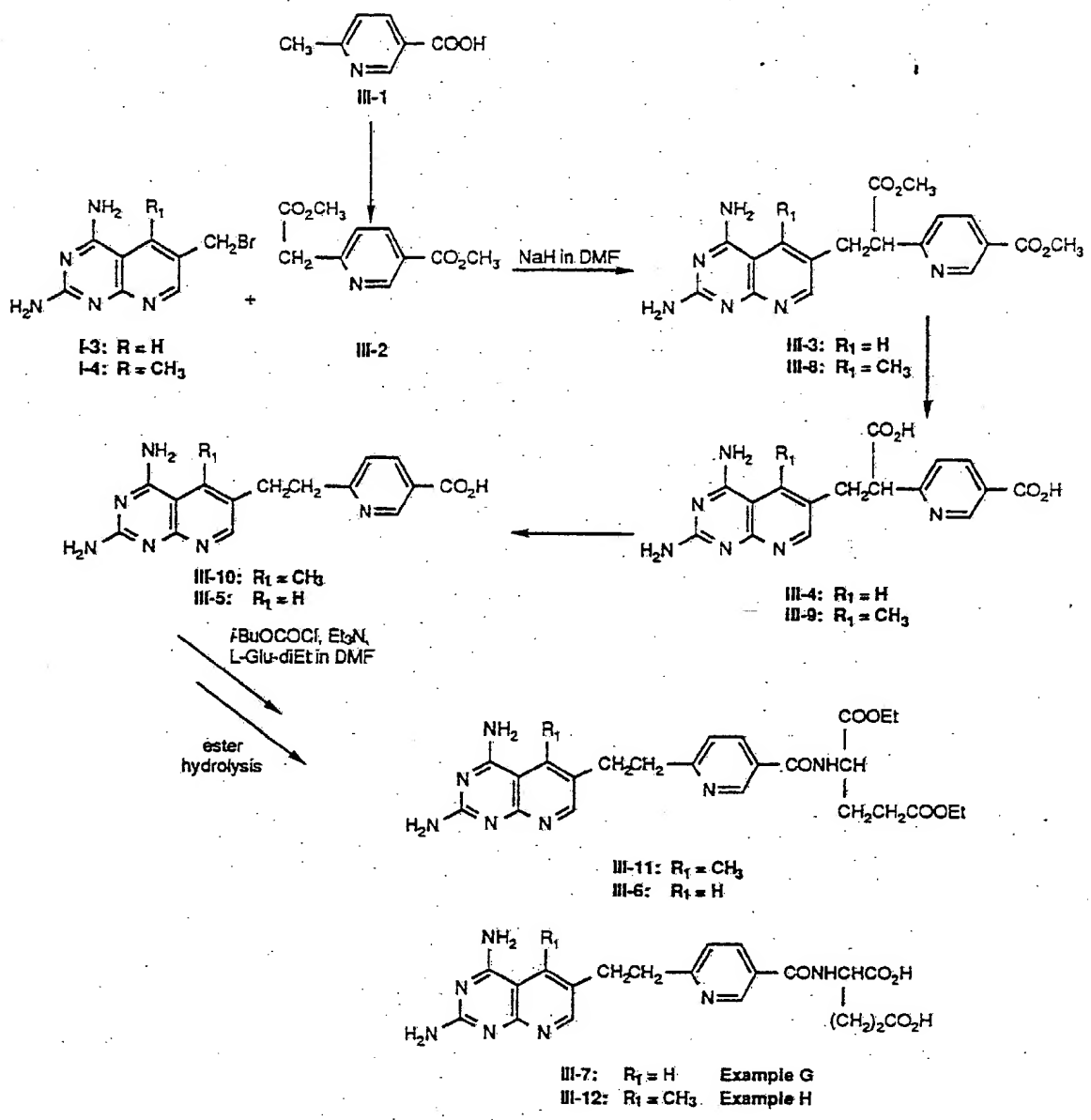
	R_1	R_2	
I-7:	H	H	Example A
I-8:	CH_3	H	Example B
I-9:	CH_3	CH_3	Example C
I-10:	CH_2CH_3	CH_3	Example D
I-11:	C_3H_7	H	Example E

Procedure II



Example F

Procedure III



Procedure IV

The following Examples A-B represent typical preparations of compounds represented by Formulae I, II, and III using Procedure I. Each compound was prepared at least twice by the procedure given. Reference numbers within the examples refer to steps or compounds prepared by steps as indicated in Procedure I or compounds listed in Table 1A.

Example A: Synthesis Of 5-Propyl-5-Deazaaminopterin (6a)

2(a) 2-Amino-6-chloro-4-propyl-3,5-pyridinedicarbonitrile. A solution of trimethylorthobutyrate (**1a**; 100 g, 0.670 mol), malononitrile (89.1 g, 1.35 mol), and pyridine (270 mL) was refluxed 1 hour. Excess pyridine was then removed by evaporation under reduced pressure (H_2O aspirator, bath to 60°C). The residue was treated with 12 *N* HCl (1.15 L) and the mixture was transferred to a 5-L three-necked flask equipped with a thermometer, condenser, and mechanical stirrer (Teflon paddle). The mixture was stirred rapidly while being heated at 85-90°C for 1 hour. Solid material formed during this time. The mixture was cooled to 20-25°C, and cold H_2O (3 L) was added. After the mixture had been kept in a refrigerator overnight, the solid was collected, washed thoroughly with H_2O and dried *in vacuo*. The product was homogeneous according to thin-layer chromatography (TLC) (EtOAc-cyclohexane, 1:1); yield 28% (42.4 g). Spectral data: mass, m/z 221, MH^+ for $C_{10}H_9ClN_4$.

3(a) 2-Amino-4-propyl-3,5-pyridinedicarbonitrile. A solution of **2a** (42.3 g, 0.193 mol) in dimethylformamide (DMF) (600 mL) and triethyl amine (Et_3N) (70 mL) containing $PdCl_2$ (1.1 g) was shaken on a Parr apparatus under H_2 at 45 psi (310.3 kPa) for 16 hours. Examination by TLC revealed conversion was incomplete. The mixture was filtered from catalyst with the aid of a little DMF. Fresh $PdCl_2$ (1.1 g) and more Et_3N (35 mL) were added to the filtrate, and hydrogenation at 45 psi (310.3 kPa) was resumed. After 3 hours, TLC showed all **2a** had been converted. The mixture was filtered and the filtrate was concentrated under reduced pressure (< 1 mm, bath 30°C) to about 75-100 mL. Dilution with cold H_2O (1 L) caused precipitation of **3a**; yield 91% (32.6 g), homogeneous by TLC. Spectral data: mas, m/z 187, MH^+ for $C_{10}H_{10}N_4$; ^1H NMR (Me_2SO-d_6) d 0.95 (t, 3, CH_3), 1.65 (m, 2, CH_2), 2.75 (t, 2, CH_2), 7.88 (br s, 2, NH_2), 8.52 (s, 1, C^6-H).

4(a) 2,4-Diamino-5-propylpyrido[2,3-d]pyrimidine-6-carbonitrile. Anhydrous guanidine•HCl (6.15 g, 0.0640 mol) and NaOMe (3.49 g, 0.0650 mol) were combined in dry 2-(2-methoxyethoxy)ethanol (270 mL), and the mixture was stirred for about 0.5 hour before it was combined with a solution of **3a** (12.0 g, 0.0640 mol) in 2-(2-methoxyethoxy)ethanol (335 mL). The stirred mixture was heated under N_2 at 150-160°C for 7 hours. This mixture was allowed to cool to about 110°C while another solution of guanidine (one-half the previous amount) in 2-(2-methoxyethoxy)ethanol was prepared. The second guanidine solution was added, and heating at 150-160°C was resumed. After 5 hours, the mixture was allowed to cool, then evaporated *in vacuo* (<1 mm) to a viscous mixture. Addition of cold H_2O (~500 mL) gave a crude solid, which was collected and dried *in vacuo*. The crude product mixture (11.4 g) was dissolved in DMF, and the solution was swirled with silica gel (about 40 g of 60-200 mesh). Evaporation *in vacuo* as before gave a solid dispersion of crude product mixture and silica gel. The dispersion was pulverized, dried further *in vacuo*, then applied to a column (9 ¥ 50-cm) of silica gel (60-200 mesh poured from $CHCl_3$). Gravity elution by $CHCl_3$-MeOH (95:5) followed,

and fractions homogeneous in **4a** (R_f ~0.55 on TLC using $CHCl_3$-MeOH, 5:1) were combined for evaporation to give pure **4a** (3.3 g, 23% yield). Spectral data: mass, m/z 229, MH^+ for $C_{11}H_{12}N_6$; ^1H NMR (Me_2SO-d_6) d 0.92 (t, 3, CH_3), 1.62 (m, 2 CH_2 CH_2CH_3), 3.20 (m, 2, $CH_2CH_2CH_3$), 6.8-7.0 (br, 2, NH_2), 7.32 (br s, 2, NH_2), 8.78 (s, 1, C^7-H).

5(a) Diethyl N-[4-[[(2,4-Diamino-5-propylpyrido[2,3-d]pyrimidin-6-yl)methyl] amino]-benzoyl]-L-glutamate. A stirred solution of **4a** (1.21 g, 5.30 mmol) and diethyl N-(4-aminobenzoyl)-L-glutamate (2.33 g, 7.23 mmol) in glacial AcOH (250 ml) containing damp Raney Ni (about 8 g) was kept under H_2 at atmospheric pressure for approximately 4 hours or until H_2 absorption from a gas buret had ceased near 240 ml. The catalyst was removed by filtration, and the filtrate was evaporated (H_2O aspirator, bath 30°C). The residue was dissolved in the minimum volume of EtOH (12-15 mL), and the stirred solution was gradually treated with 3% Na_2CO_3 solution to pH 7.8. The resulting crude product mixture was collected with the aid of cold H_2O, dried, and dispersed onto silica gel (60-200 mesh) as described above for precursor **4a**. The dispersion was applied to a silica gel column (5 ¥ 50-cm) poured from $CHCl_3$. Elution by gravity flow with $CHCl_3$-MeOH (95:5) followed. After TLC showed all diethyl N-(4-aminobenzoyl)-L-glutamate and minor contaminants more mobile than **5a** had been eluted, the system was switched to 85:15 $CHCl_3$-MeOH. Fractions homogeneous with respect to **5a** (R_f ~0.5 using $CHCl_3$-MeOH, 3:1) were combined and evaporated to give pure **5a** in 16% yield (470 mg). Spectral data: Mass, m/z 538, MH^+; ^1H NMR d 0.92 (t, 3, CH_3), 1.12-1.22 (2 t, 6, CH_3CH_2O overlapping), 1.54 (m, 2, $CH_2CH_2CH_3$), 1.98 and 2.06 (2 m, 2, $CHCH_2CH_2$, nonequivalent), 2.42 (t, 2, CH_2CH_2CO), 3.02 (t, 2, $CH_2CH_2CH_3$), 4.00-4.14 (br m, 4, CH_3CH_2O overlapping), 4.32 (d, 2, CH_2NH), 4.38 (m, 1, CONHCH), 6.35 (br s, 2, NH_2), 6.56 (t, 1, CH_2NH), 6.66 and 7.68 (2 d, 4, C_6H_4), 7.05 (s, 2, NH_2), 8.25 (d, 1, CONH), 8.52 (s, 1, C^7-H). Anal. Calcd. for $C_{27}H_{35}N_7O_5 \cdot 0.5\ H_2O$; C, 59.33; H, 6.64; N, 17.94. Found: C, 59.24, 59.55, H, 6.56, 6.49; N, 17.68, 17.71.

6(a) N-[4-[[(2,4-Diamino-5-propylpyrido[2,3-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (5-Propyl-5-deazaaminopterin). A solution of **5a** (400 mg, 0.732 mmol) in MeOH (800 mL) was treated with 1N NaOH (1.7 ml), and the solution was kept at 20-25°C for 64 hours. MeOH was removed by evaporation under reduced pressure (H_2O aspirator, bath 20-25°C). Solution did not occur when the residue was treated with H_2O (50 ml), indicating that unchanged **6a** was still present. The mixture was again made a clear solution by adding MeOH (300 ml) back to the aqueous suspension. The solution was left 48 hours longer at 20-25°C when high performance liquid chromatography (HPLC) [Piper et al., J. Med. Chem., 29, 1080 (1986)] indicated that virtually all **5a** had been converted to either **6a** disodium salt or a monosodium salt. MeOH was again removed, and the aqueous residue (adjusted to 50 ml) was treated with more 1N NaOH (0.8 ml). After 4-5 more days at 20-25°C, HPLC showed that conversion to **6a** was complete. The solution was clarified (Norit, Celite) and treated with 1N HCl to precipitate **6a** at pH 3.8 as a pale beige solid; yield, 69% (260 mg), hydrated as indicated

below. Assay by HPLC showed the product to be homogeneous. Spectral data: Mass, m/z 482, MH^+; UV, l_{max} 228 nm (e 38,300), 299 (22,500) at pH 1; 225 nm (e 34,400), 284 (26,500) at pH 7; 225 nm (e 32,000), 284 (26,700) at pH 13; ^1H NMR (Me_2SO-d6) d 0.92 (t, 3, CH_3), 1.56 (m, 2, $CH_2CH_2CH_3$), 1.96 and 2.02 (two m, 2, $CHCH_2CH_2$, nonequivalent), 2.32 (t, 2 CH_2CH_2CO), 3.4 (t, 2, $CH_2CH_2CH_3$), 4.30 (d, 2 CH_2NH), 4.32 (m, 1, CONHCH), 6.54 (t, 1, CH_2NH), 6.64 and 7.66 (2 d, 4, C_6H_4), 6.65 (br, 2 NH_2), 7.24 (br, 2, NH_2), 8.06 (d, 1, CONH), 8.52 (s, 1, C^7-H). Anal. Calcd. for $C_{23}H_{27}N_7O_5 \cdot 2\ H_2O$: C, 53.38; H, 6.04; N, 18.94. Found: C, 53.72, 53.61; H, 5.86, 5.84; N, 18.74, 18.75.

Example B: Synthesis Of 5-Butyl-5-Deazaaminopterin

2(b) 2-Amino-6-chloro-4-butyl-3,5-pyridinedicarbonitrile. A solution of trimethyl orthovalerate (**1b**; 20.1 g, 0.124 mol) and malononitrile (16.4 g, 0.248 mol) in pyridine (50 mL) was refluxed 45 min, cooled, and evaporated. The residue was stirred with 12N HCl (210 mL) at 85°C (bath temp) for 45 min to give **2b** as an insoluble solid. After dilution with H_2O (100 mL), the mixture was chilled and the solid collected to give **2b** in 27% yield (7.96 g); homogeneous by TLC (cyclohexane-EtOAc, 1:1). Spectral data: Mass, m/z 235, MH^+ for $C_{11}H_{11}ClN_4$.

3(b) 2-Amino-4-butyl-3,5-pyridinedicarbonitrile. Hydrogenolysis of **2b** (7.32 g, 31.2 mmol) in DMF (88 ml) containing $PdCl_2$) and Et_3N (9 ml) was conducted in a Parr shaker at 40 psi for 16 hours. Examination by TLC revealed absence of **2b**. The catalyst was removed by filtration, and the filtrate was diluted with H_2O to cause precipitation of **3b**. The collected solid was reprecipitated from a Norit-treated and filtered (Celite) solution in DMF (120 ml) by adding H_2O; yield, 90% (5.6 g); homogeneous by TLC (cyclohexane-ETOAc, 1:1). Spectral data: Mass, m/z 201, MH^+ for $C_{11}H_{12}N_4$; ^1H NMR (Me_2SO-d_6) d 0.92 (t, 3, CH_3), 1.38 (m, 2, $CH_2CH_2CH_2CH_3$), 1.58 (m, 2, $CH_2CH_2CH_2CH_3$), 2.75 (t, 2, $CH_2CH_2CH_2CH_3$), 7.88 (br, 2, NH_2), 8.52 (s, 1, C^6-H).

4(b) 2,4-Diamino-5-butylpyrido[2,3-d]pyrimidine-6-carbonitrile. The annulation of **3b** with guanidine was conducted as described for the conversion of **3a** to **4a**. A typical yield of pure **4b** after column chromatographic purification (as described for **4a**) was 20%; homogeneous by TLC ($CHCl_3$-MeOH, 7:1). Spectral data: Mass, m/z 342, MH^+ for $C_{12}H_{14}N_6$; ^1H NMR d 0.88 (t, 3, CH_3), 1.34 (m, 2, $CH_2CH_2CH_2CH_3$), 1.56 (m, 2, $CH_2CH_2CH_2CH_3$), 3.24 (t, 2, $CH_2CH_2CH_2CH_3$), 6.9 (br, 2, NH_2), 7.34 (br, 2, NH_2), 8.76 (s, 1, C^7-H).

5(b) Diethyl N-[4-[[(2,4-Diamino-5-butylpyrido[2,3-d]pyrimidin-6-yl)methyl]amino]-benzoyl-L-glutamate. Reductive condensation of **4b** with diethyl N-(4-aminobenzoyl)-L-glutamate was conducted as described for **5a**. Pure **5b** was also isolated as described for **5a**. A typical yield of pure **5b** was 15%. Spectral data: Mass, m/z 552, MH^+ for $C_{28}H_{37}N_7O_5$; ^1H NMR (Me_2SO-d_6) d 0.85 (t, 3, CH_3), 1.12-1.22 (2 t, 6, CH_3CH_2O, overlapping), 1.35 (m, 2,

$CH_2CH_2CH_2CH_3$), 1.52 (m, 2, $CH_2CH_2CH_2CH_3$), 1.98 and 2.05 (2 m, 2, $CHCH_2CH_2$, nonequivalent), 2.42 (t, 2, CH_2CH_2CO), 3.04 (t, 2, $CH_2CH_2CH_2CH_3$), 4.0-4.15 (br m, 4, CH_3CH_2O, overlapping), 4.30 (d, 2, CH_2NH), 4.38 (m, 1, CONHCH), 6.24 (br s, 2, NH_2), 6.52 (t, 1, CH_2NH), 6.66 and 7.68 (2 d, 4, C_6H_4), 6.92 (br s, 2, NH_2), 8.24 (d, 1, CONH), 5.40 (s, 1, C^7-H).

6(b) N-[[(2,4-Diamino-5-butylpyrido[2,3-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (5-butyl-5-deazaaminopterin. A solution of **5b** (50 mg, 0.091 mmol) in MeOH (150 ml) treated with 1N NaOH (0.24 ml) was kept at 20-25°C for 4 days. MeOH was evaporated *in vacuo* (H_2O aspirator, bath 25°C), and the residue was dissolved in H_2O (3 mL). After 30 hours at 20-25°C, the solution was carefully treated with 1N HCl to pH 3.8, where **6b** precipitated; yield, 51% (24 mg). Assay by HPLC showed the product to be of 99.4% purity; spectral data: Mass, m/z 496, MH^+; UV, l_{max} 300 nm (e 23,900) at pH 1; 287 nm (e 25,900) at pH 7; 287 nm (e 26,100) at pH 13; ^1H NMR (Me_2SO-d6) 0.85 (t, 3, CH_3), 1.35 (m, 2, $CH_2CH_2CH_2CH_3$), 1.52 (m, 2, $CH_2CH_2CH_2CH_3$), 1.96 and 2.02 (2 m, 2, $CHCH_2CH_3$, nonequivalent), 2.32 (t, 2, CH_2CH_2CO), 3.06 (t, 2, $CH_2CH_2CH_2CH_3$), 4.3 (m, 3, CH_2NH overlapping with CONHCH), 6.54 (t, 1, CH_2NH), 6.66 and 7.66 (2 d, 4, C_6H_4), 7.18 (br s, 2, NH_2), 8.08 (d, 1, CONH), 8.54 (s, 1 C^7H). Anal. Calcd. for $C_{24}H_{29}N_7O_5 \cdot 1.5\ H_2O$: C, 55.17; H, 6.17; N, 18.76. Found: C, 55.12; H, 6.03; N, 18.59.

The synthesis of the compounds of Formula III, wherein A is CH and wherein R_2 is H, methyl or ethyl, is described by Taylor et al., J. Med. Chem., 263, 914 (1985), and Taylor et al., U.S. Patent Nos. 4,845,216, issued July 4, 1989; and 4,684,653, issued August 4, 1987; and PTC International Publication No. WO86105181 dated September 12, 1986. These references indicate these compounds have utility as antineoplastic agents, and so compounds wherein R_2 is three or more carbon atoms would be expected to have similar utility.

The Examples A'-H' which follow illustrate application of Procedures II, III, and IV to the preparation of specific compounds of Formulae I, IV, and V, and represent preferred embodiments of the invention. Reference numbers within the examples refer to steps or compounds prepared by steps described in Procedures II, III, and IV or compounds listed in Table 1B.

Example A': Synthesis Of Compound 1, Table 1B By Procedure II

N-(5-Aminothiophene-2-carbonyl)-L-glutamic Acid Diethyl Ester (**I-1**). This compound was prepared by the method of Marsham et al., J. Med. Chem., 34, 1594 (1991).

6-(Bromomethyl)-2,4-diaminopyrido[2,3-d]pyrimidine (**I-3**). This intermediate was prepared from 2,4-diaminopyrido[2,3-d]pyrimidine-6-methanol by the procedure of Piper et al., J. Med. Chem., 35, 332 (1992). This particular preparation analyzed for a 1.75 HBr • 0.25 CH_3COOH salt (formula wt. 406.7). The material was suitable for conversions to compounds **I-7, II-7, and III-7** in Table 1B.

N-[5-[[(2,4-Diaminopyrido[2,3-d]pyridin-6-yl)methyl]amino]thiophene-2-carbonyl]-L-glutamic Acid (**I-7**). The bromomethyl compound **I-3** and the sidechain precursor **I-1** (1.2 mmol of each) were stirred with $CaCO_3$ (2.4 mmol) in Me_2NAc (15 mL) at 20-25°C for 4 days. The mixture was filtered, and the clear filtrate was added dropwise to excess 2.5% $NaHCO_3$ solution with stirring. The precipitate that formed was collected, dried, and chromatographed on silica gel with elution by $CHCl_3$-MeOH (2:1) to give essentially pure diethyl ester; mass spectrum, m/e 502 (MH+ for $C_{22}H_{27}N_7O_5S$); predominantly one peak (>92%) by HPLC; yield 91 mg (15%). For hydrolysis, the ester (90 mg) was stirred with 1 N NaOH (1.8 mL) for 5 h (solution occurred after 1.5 h). Acidification to pH 3.8 caused precipitation of the product; yield 67 mg (75%) mass spectrum, m/e 446, MH+ for $C_{18}H_{19}N_7O_5S$.

Example B': Synthesis Of Compound 2, Table 1B By Procedure II

6-(Bromomethyl)-2,4-diamino-5-methylpyrido[2,3-d]pyrimidine (**I-4**). 2,4-Diamino-5-methylpyrido[2,3-d]pyrimidine-6-methanol, [Piper et al., J. Med. Chem., 35, 3002 (1992)] (4.5 g, 22.0 mmol) was dissolved in glacial AcOH (200 mL) at 95°C. The solution was cooled to 25°C, then treated with stirring with 30% dry HBr in AcOH (400 mL). When addition was complete, a clear solution remained. The flask was stoppered securely, and the solution was kept at 20-25°C before it was added to Et_2O (2.2 L) with stirring. The precipitate that formed was collected under N_2, washed with Et_2O, and dried in vacuo (P_2O_5 and NaOH pellets); yield 9.5 g of **I-4** hydrobromide solvated by AcOH; yield 99% (based on formulation shown below), mass spectrum, m/e 268 and 270, MH+ for $C_9H_{10}BrN_5$; 1H NMR (Me_2SO-d6) d 2.78 (s, 3, CH_3), 4.93 (s, 2, CH_2Br), 8.17 (s, 2, NH_2), 8.75 (s, 1, C_7H), 9.32 (s, 2, NH_2); solvation by CH_3CO_2H evidenced by methyl-group singlet at d 1.90 whose integral height is one-half that of the CH_3 group of **I-4**. Thus the molar ratio of I-4 to CH_3CO_2H is 1:0.5. Anal. Calcd for $C_9H_{10}BrN_5 \cdot 1.7HBr \cdot 0.5CH_3CO_2H$ (formula wt. 435.7): C, 27.57; H, 3.17; N, 16.07. Found: C, 27.53, H, 3.37; N, 16.11.

N-[5-[[(2,4-Diamino-5-methylpyrido[2,3-d]pyrimidin-6-yl)methyl]amino]thiophene-2-carbonyl]-L-glutamic Acid (**I-8**). Bromomethyl compound **I-4** (3.5 g, 8.0 mmol), sidechain precursor **I-1** (2.7 g, 8.2 mmol), and $CaCO_3$ (1.63 g, 16.3 mmol) were combined in Me2NAc (50 mL). The stirred mixture was warmed briefly (5-10 min.) at 70°C. Reactants other than $CaCO_3$ dissolved readily. The mixture was stirred at 20-25°C in a stoppered flask under N_2 for 7 days. Inorganic matter was filtered off, and silica gel (25 g) was added to the filtrate. The slurry was evaporated in vacuo, and the dispersion was pulverized for application on a silica gel column (4 x 50 cm). Elution by $CHCl_3$-MeOH (95 : 5) removed front-running impurities. The product was eluted with $CHCl_3$-MeOH (85 : 15). Appropriate fractions were combined and evaporated to give the diethyl ester of I-8 (3.4 g, 81% yield). Mass spectrum, m/e 516, MH+ for $C_{23}H_{29}N_7O_5S$. I-8 diethyl ester (258 mg, 0.50 mmol) was stirred 3 hours under N_2 with 1N NaOH (5.0 mL). The resulting clear solution was treated with 2N HCl to pH 3.5 to give **I-8**; yield 46% (0.11 g). Mass spectrum, 460, MH+; 1H NMR (Me_2SO-d6) d 1.88, 1.98 (two m, 2, $CHCH_2$, nonequivalent), 2.30 (t, 2, CH_2CO), 2.66 (s, 3, CH_3), 4.25 (m, 1, $CHCH_2$), 5.94 (d, 1, 4-ArH adjacent to 5-ArNH), 6.68 (s, 2, NH_2), 7.2-7.35 (m, 3, NH_2 overlapping 5-ArNH), 7.48 (d, 1, 3-ArH), 7.98 (d, 1, CONH), 8.52 (s, 1, C7-H). Anal. Calcd for $C_{19}H_{21}N_7O_5S \cdot 2.8H_2O$: C, 44.74; H, 5.26; N, 19.23. Found: C, 44.78; H, 5.18; N, 19.49.

Example C': Synthesis Of Compound 12, Table IB, By Procedure II

N-[5-(Methylamino)thiophene-2-carbonyl]-L-glutamic Acid Diethyl Ester (**I-2**). A solution containing **I-1** (1.78 g, 5.42 mmol), $(i\text{-}Pr)_2NEt$ (1.0 mL, 0.74 g, 5.7 mmol), and Me2SO4 (0.59 mL, 0.79 g, 6.2 mmol) in N,N-dimethylformamide (DMF, 20 mL) was warmed at 60°C for 2 h, then left at 20-25°C for 42 hours. The solution was evaporated in vacuo (1 mm, bath 25-30°C), and the residue was dissolved in EtOAc-cyclohexane (1:1 by volume) for application to a silica gel column. Elution by the same solvent system afforded fractions homogeneous by TLC in **I-2**. Fractions were combined and evaporated to afford 16% yield (287 mg) of I-2 as an amber oil. Mass spectrum, m/e 343, MH+ for $C_{15}H_{22}N_2O_5S$.

N-[5-[[(2,4-Diamino-5-methylpyrido[2,3-d]pyrimidin-6-yl)methyl]methylamino]thiophene-2-carbonyl]-L-glutamic Acid (**I-9**). This compound was prepared from **I-4** (0.91 mmol) and sidechain precursor I-2 (0.96 mmol) by essentially the same procedure as described above for the preparation of **I-8**. After filtration, the reaction solution was evaporated (<1 mm, bath to 40°C). The residue was dissolved in $CHCl_3$-MeOH (6 : 1) for application to a silica gel column. Elution by $CHCl_3$-MeOH (6 : 1) gave fractions homogeneous by TLC ($CHCl_3$-MeOH, 4 : 1; Rf ~0.5) which were combined and evaporated to give the diethyl ester of **I-9**; yield 27% (132 mg), Mass spectrum, m/e 530, MH+ for $C_{24}H_{31}N_7O_5S$. This sample was hydrolyzed as described for **I-8** to give pure I-9•3H_2O in 80% yield (109 mg), Mass spectrum, m/e 474, MH+; 1H NMR (Me_2SO-d6) d 1.90, 200. (two m, 2, $CHCH_2$ nonequivalent), 2.32 (t, 2, CH_2CO), 2.60 (s, 3, 5-CH_3), 2.87 (s, 3, CH_3N), 4.30 (m, 1, $CHCH_2$),

4.50 (s, 2, CH_2N), 6.05 (d, 1, 4-ArH adjacent to 5-ArN), 6.68 (s, 2, NH_2), 7.32 (s, 2, NH_2), 7.58 (d, 1, 3-ArH), 8.06 (d, 1, CONH), 8.35 (s, 1, C7H). Anal. Calcd for $C_{20}H_{23}N_7O_5S \cdot 3H_2O$: C, 45.53; H, 5.54; N, 18.59. Found: C, 45.60; H, 5.28; N, 18.36.

Example D': Synthesis Of Compound 19, Table IB, By Procedure II

6-(Bromomethyl)-2,4-diamino-5-ethylpyrido[2,3-d]pyrimidine (**I-5**). 2,4-Diamino-5-ethylpyrido[2,3-d]pyrimidine-6-methanol (2.80 g, 12.8 mmol), prepared as described for the 5-CH_3 homolog [Piper et al., J. Med. Chem., 35, 3002 (1992)], was converted to **I-5** as described above for **I-4**; yield 5.3 g, Mass spectrum, m/e 282 and 284, MH+ for $C_{10}H_{12}BrN_5$; 1H NMR (MeSO-d6) d 1.24 (t, 3, CH_3), 3.24 (q, 2, CH_2), 4.94 (s, 2, CH_2Br), 8.14 (s, 2, NH_2), 8.80 (s, 1, C7-H), 9.22 (s, 2, NH_2); solvation by CH_3CO_2H is evidenced by methyl-group singlet at d 1.92 whose integral height is one-third that of the CH_3 group of **I-5**. Based on the results, the formulation for the product would be **I-5**•1.4HBr•0.33CH_3CO_2H (formula wt. 416.7).

N-[5-[[(2,4-Diamino-5-ethylpyrido[2,3-d]-pyrimidin-6-yl)methyl]methylamino]-thiophene-2-carbonyl]-L-glutamic Acid (**I-10**). Alkylation of **I-2** by **I-5** (0.84 mmol of each) was done as described under the preparations of **I-8** and **I-9**. Purification of the ester produced was done as described for **I-9** with $CHCl_3$-MeOH (7 : 1) used as eluant. The yield of pure diethyl ester of **I-10** was 27% (124 mg); mass spectrum, m/e 544, MH+ for $C_{25}H_{33}N7O_5S$. This sample was hydrolyzed as described for **I-8** (or **I-9**) to give **I-10**•3H_2O in 82% yield (102 mg), Mass spectrum, m/e MH+; 1H NMR (Me_2SO-d6) d 1.16 (t, CH_3CH_2), 1.90, 2.00 (two m, 2, $CHCH_2$ nonequivalent), 2.30 (t, 2, CH_2CO), 2.86 (s, 3, CH_3N), 3.00 (q, 2, CH_3CH_2), 4.28 (m, $CHCH_2$), 4.52 (s, 2, CH_2N), 6.05 (d, 1, 4-ArH adjacent to 5-ArN), 6.60 (s, 2, NH_2), 7.22 (s, 2, NH_2), 7.61 (d, 1, 3-ArH), 8.06 (d, 1, CONH), 8.40 (s, 1, C7H). Anal. Calcd for $C_{21}H_{25}N_7O_5S \cdot 3H_2O$: C, 46.57; H, 5.77; N, 18.10. Found: C, 46.55,; H, 5.52; N, 18.15.

Example E': Synthesis Of Compound 21, Table IB, By Procedure II

Preparation of 6-Bromomethyl-2,4-diamino-5-propylpyrido(2,3-d)pyrimidine (**I-6**). 2-Amino-6-chloro-4-propyl-3,5-pyridinedicarbonitrile. A solution of trimethyl orthobutyrate (1a; 100 g, 0.670 mol), malononitrile (89.1 g, 1.35 mol), and pyridine (270 mL) was refluxed 1 hour. Excess pyridine was then removed by evaporation under reduced pressure (H_2O aspirator, bath to 60°C). The residue was treated with 12 N HCl (1.15 L) and the mixture was transferred to a 5-L three-necked flask equipped with a thermometer, condenser, and mechanical stirrer (Teflon paddle). The mixture was stirred rapidly while being heated at 85-90°C for 1 hour. Solid material formed during this time. The mixture was cooled to 20-25°C, and cold H_2O (3 L) was added. After the mixture had been kept in a refrigerator overnight, the solid was collected, washed thoroughly with H_2O and dried in vacuo. The product was homogeneous according to TLC (EtOAc-cyclohexane, 1:1); yield 28% (42.4 g). Spectral data: mass, m/z 221, MH+ for $C_{10}H_9ClN_4$.

2-Amino-4-propyl-3,5-pyridinedicarbonitrile. A solution of **2a** (42.3 g, 0.193 mol) indimethylformamide (DMF, 600 mL) and triethyl amine (Et_3N, 70 mL) containing $PdCl_2$ (1.1 g) was shaken on a Parr apparatus under H_2 at 45 psi (310.3 kPa) for 16 hours. Examination by TLC revealed conversion was incomplete. The mixture was filtered from catalyst with the aid of a little dimethyl formamide (DMF). Fresh $PdCl_2$ (1.1 g) and more Et_3N (35 mL) were added to the filtrate, and hydrogenation at 45 psi (310.3 kPa) was resumed. After 3 hours, TLC showed all **2a** had been converted. The mixture was filtered and the filtrate was concentrated under reduced pressure (< 1 mm, bath 30°C) to about 75-100 mL. Dilution with cold H_2O (1 L) caused precipitation of **3a**; yield 91% (32.6 g), homogeneous by TLC. Spectral data: mas, m/z 187, MH+ for C10H10N4; 1H NMR (Me2SO-d6) d 0.95 (t, 3, CH3), 1.65 (m, 2, CH_2), 2.75 (t, 2, CH_2), 7.88 (br s, 2, NH_2), 8.52 (s, 1, C_6-H).

2,4-Diamino-5-propylpyrido[2,3-d]pyrimidine-6-carbonitrile. Anhydrous guanidine•HCl (6.15 g, 0.0640 mol) and NaOMe (3.49 g, 0.0650 mol) were combined in dry 2-(2-methoxyethoxy)ethanol (270 mL), and the mixture was stirred for about 0.5 hour before it was combined with a solution of **3a** (12.0 g, 0.0640 mol) in 2-(2-methoxyethoxy)ethanol (335 mL). The stirred mixture was heated under N_2 at 150-160°C for 7 hours. This mixture was allowed to cool to about 110°C while another solution of guanidine (one-half the previous amount) in 2-(2-methoxyethoxy)ethanol was prepared. The second guanidine solution was added, and heating at 150-160°C was resumed. After 5 hours, the mixture was allowed to cool, then evaporated in vacuo (<1 mm) to a viscous mixture. Addition of cold H_2O (~500 mL) gave a crude solid, which was collected and dried in vacuo. The crude product mixture (11.4 g) was dissolved in DMF, and the solution was swirled with silica gel (about 40 g of 60-200 mesh). Evaporation in vacuo as before gave a solid dispersion of crude product mixture and silica gel. The dispersion was pulverized, dried further in vacuo, then applied to a column (9 ¥ 50-cm) of silica gel (60-200 mesh poured from $CHCl_3$). Gravity elution by $CHCl_3$-MeOH (95:5) followed, and fractions homogeneous in **4a** (Rf ~0.55 on TLC using $CHCl_3$-MeOH, 5:1) were combined for evaporation to give pure **4a** (3.3 g, 23% yield). Spectral data: mass, m/z 229, MH+ for $C_{11}H_{12}N_6$; 1H NMR (Me_2SO-d6) d 0.92 (t, 3, CH_3), 1.62 (m, 2 $CH_2CH_2CH_3$), 3.20 (m, 2, $CH_2CH_2CH_3$), 6.8-7.0 (br, 2, NH_2), 7.32 (br s, 2, NH_2), 8.78 (s, 1, C_7-H).

2,4-Diamino-5-propylpyrido[2,3-d]pyrimidine-6-carboxaldehyde. Raney Ni (1.2 g damp) was added with the aid of 95-97% HCO_2H (7 mL) to a solution of 2,4-diamino-5-propylpyrido[2,3-d]pyrimidine-6-carbonitrile (500 mg, 2.19 mmol) in 95-97% HCO_2H (5 mL). The stirred mixture was heated at 75-80°C for 1.5 h. Raney Ni was removed by filtration, and the filtrate was evaporated. The residue was dissolved in hot H_2O (20 mL), and the solution was filtered, then cooled, and treated with concentrated NH_4OH to pH 7 to cause solid to precipitate. The mixture was kept at 0-5°C overnight before the solid was collected and washed with cold H_2O. This crude material was applied to a silica gel column and eluted with $CHCl_3$-MeOH (7:1). Evaporation of the product fractions afforded the aldehyde; yield 8% (41 mg). Anal.

Calcd. for $C_{11}H_{13}N_5O \cdot 0.2H_2O$: C, 56.26; H, 5.75; N, 29.82. Found: C, 56.26; H, 5.70, N, 29.72. MS, m/e 232, MH+; UV, lmax (e ¥ 10-3) in 0.1N HCl, 235 (22.9), 257 (19.5), 316 (9.15); pH 7 buffer, 234 (15.7), 265 (17.4), 318 (11.0), 346 (12.2); in 0.1N NaOH, 234 (17.5), 265 (16.6), 347 (12.8); 1H NMR (Me_2SO-d6), d 0.92 (t, 3, CH_3), 1.55 (sext, 2, $CH_2CH_2CH_3$), 3.46 (t, 2, $CH_2CH_2CH_3$), 6.84 (br s, 2, NH_2), 7.30 (s, 2, NH_2), 8.88 (s, 1, C_7-H), 11.0 (s, 1, CHO).

2,4-Diamino-5-propylpyridol[2,3-d]pyrimidine-6-methanol. The aldehyde (95 mg, 0.41 mmol) was stirred with MeOH (20 mL), and the near-solution was treated with 3 portions of $NaBH_4$ (17 mg total, 0.45 mmol) added at 15-min intervals. Complete solution occurred after the first addition of $NaBH_4$. The solution was left at 20-25°C for 1 h. The solution was treated with H_2O (1 mL), neutralized (to pH 7) with glacial AcOH, and evaporated to near dryness. Solid residue was stirred with a little cold H_2O (~1 mL), collected, and dried to give a first crop of 6-hydroxymethyl compound; yield 31% (30 mg); MS, m/e 234, MH+ for $C_{11}H_{15}N_5O$: 1H NMR (Me_2SO-d6), d 0.96 (t, 3, CH_3), 1.56 (sext, 2, $CH_2CH_2CH_3$), 3.06 (t, 2, $CH_2CH_2CH_3$), 4.52 (s, 2, CH_2OH), 6.22 (s, 2, NH_2), 6.90 (s, 2, NH_2), 8.48 (s, 1, C_7-H). The filtrate from this sample was evaporated to dryness, and the residue was extracted several times with boiling EtOAc to provide another crop of hydroxymethyl compound (80 mg), MS m/e 234.

6-(Bromomethyl)-2,4-diamino-5-propylpyrido[2,3-d]pyrimidine **(I-6)**. The hydroxymethyl compound was treated with dry HBr in AcOH as reported for the methyl homolog **(I-4)**. Addition of Et_2O caused precipitation of yellow solid which was collected with the aid of Et_2O and dried in vacuo to afford the HBr salt of the product; MS m/e 296 and 298, MH+ for $C_{11}H_{14}BrN_5$, but with higher mass peaks present. The 1H NMR spectrum of the product mixture showed expected singlets due to the CH_2Br (4.92) and the C_7-H (8.78). Relative integral values suggested about 10 mole-percent of the product.

N-[5-[[(2,4-Diamino-5-propylpyrido[2,3-d]pyrimidin-6-yl)methyl]amino]thiophene-2-carbonyl]L-glutamic Acid **(I-11)**. A mixture of the crude bromomethyl preparation described above (0.55 g), diethyl N-(5-aminothiophene-2-carbonyl)-L-glutamate (0.40 g, 1.2 mmol) and $CaCO_3$ (160 mg) in Me_2NAc (7 mL) was stirred at 20-25°C for 4 days. Insoluble material was removed by filtration, and the filtrate was evaporated to dryness. The residue was chromatographed on silica get with elution by $CHCl_3$-MeOH (5:1) to give fractions homogeneous in the desired product. Evaporation of the pooled fractions gave the diethyl ester as a pale-orange solid (20 mg); MS m/e 545, MH+ for $C_{25}H_{33}N_7O_5S$. For ester hydrolysis, this sample was dissolved in 1 N NaOH (0.37 mL), and the solution was kept at 20-23°C for 5 h. The solution was clarified (Celite mat), then acidified to pH 3.8-4.0 with 2 N HCl. After the mixture had been refrigerated, the solid was collected and dried; yield 6 mg. MS, m/e 488, MH+ for $C_{21}H_{25}N_7O_5S$.

Example F': Synthesis Of Compound 71, Table IIB, By Procedure III

2-Carboxythiophene-5-acetic Acid Dimethyl Ester (II-2). Freshly distilled diisopropylamine (24.6 g, 0.24 mol) in 250 mL of dry tetrahydrofuran (THF) was cooled to 0°C under argon then treated dropwise with 98 mL (0.24 mol) of 2.5M BuLi in hexane. After 1 h, the LDA solution was added dropwise with stirring to a -30° mixture of 15.0 g (0.11 mol) of 5-methylthiophene-2-carboxylic acid (II-1) in 300 mL of dry THF. The temperature of the resulting red solution was allowed to rise to 0°C and was so maintained for another 2 h. Carbon dioxide was bubbled through the solution to produce a yellow precipitate. The mixture was stirred at ambient temperature for 2 h and filtered. The yellow filter cake was suspended in 300 mL of MeOH, and the mixture was cooled to 0°C and treated with 100 mL of MeOH saturated with dry HCl. The mixture was stirred at room temperature for 72 h, concentrated in vacuo and the residue was partitioned between Et_2O (500 mL) and 250 mL of sat'd $NaHCO_3$. The Et_2O extract was washed with H_2O (3 ¥ 250 mL), dried over $MgSO_4$ and evaporated to leave a dark oil (15 g). Chromatography on flash silica gel (EtOAc-hexane, 1:19) gave 11.4 g of the product (51%) as a white, waxy solid; NMR ($CDCl_3$) d 7.61 (d, 1H, 3-H); 6.90 (d, 1H,. 4-H); 3.87 (m, 5H, $ArCOOCH_3$ + CH_2); 3.82 (s, 3H, CH_2COOCH_3); Anal. ($C_9H_{10}O_4S$); Calc: C, 50.5; H, 4.71; Found: C, 50.6; H, 4.79.

5-[1-Carbomethoxy-2-(2,4-diamino[2,3-d]pyrimidin-6-yl)ethyl]thiophene-2-carboxylic Acid Methyl Ester (II-3). The sodio derivative of 2-carbomethoxythiophene-5-acetic acid methyl ester (II-2) (12.0 mmol) was preparaed in DMF using NaH (480 mg of 60% dispersion in oil, 12.0 mmol). The mixture was treated with a solution of I-3 (1.6 g, 4.0 mmol) in 10 mL of DMF at -25°C. The mixture was kept at -10°C for one hour and then stirred for one hour at room temperature, followed by neutralization with solid CO_2. Silica gel was added followed by evaporation in vacuo. The solid residue was applied to a silica gel column and the product eluted with $CHCl_3$-MeOH (9:1) to afford II-3 in 23% yield (350 mg); mass spectrum, m/e 388, MH+ for $C_{17}H_{17}N_5O_4S$.

5-[1-Carboxy-2-(2,4-diamino[2,3-d]pyrimidin-6-yl)ethyl]thiophene-2-carboxylic Acid (II-4). The ester II-3 (350 mg) was hydrolyzed by 4N NaOH (0.5 mL) in dimethylsulfoxide (DMSO) for 20 hours followed by removal of DMSO at 40° in vacuo. Precipitation of II-4 from a solution of its Na salt in H_2O by treatment with 1N HCl to pH 4.0 gave a gel-like precipitate. The gel became particulate solid after the mixture had been frozen solid and then allowed to thaw. The solid was then easily collected by filtration; yield 85% (275 mg); mass spectrum, m/e 360, MH+ for $C_{15}H_{13}N5O_4S$. Assay by HPLC, >93% purity.

5-[2-(2,4-Diamino[2,3-d]pyrimidin-6-yl)ethyl]thiophene-2-carboxylic Acid (**II-5**). A solution of **II-4** (235 mg, 0.65 mmol) in DMSO (8 mL) was kept at 120-125°C for 20 min. Removal of DMSO in vacuo (<1 mm, bath to 40°C) gave II-5; mass spectrum, m/e 316, MH+ for $C_{14}H_{13}N_5O_2S$. This material was used directly for conversion to **II-6**.

N-[5-[2-(2,4-Diamino[2,3-d]pyrimidin-6-yl)ethyl]thiophene-2-carbonyl]-L-glutamic Acid Diethyl Ester (**II-6**). A stirred mixture of **II-5** (210 mg, 0.66 mmol) in DMF (25 mL) was treated with Et_3N (0.37 mL, 26 mg, 2.64 mmol) followed by i-BuOCOCl (0.09 mL, 92 mg, 0.66 mmol), then stirred at 20-23°C for 15 min before diethyl L-glutamate•HCl (158 mg, 0.66 mmol) was added. Three more additions of i-BuOCOCl (0.33, 0.17, and 0.17 mmol) were made at intervals of 15 min, and each was followed 1 min later by an equimolar amount of diethyl L-glutamate•HCl. The course of the conversion was followed by TLC ($CHCl_3$-MeOH, 3 : 1), and a chromatogram observed 2 h after the final addition revealed one major UV-absorbing spot. DMF was then removed (<1 mm, 25-30°C). The residue was dissolved in MeOH, and the solution was treated with silica gel (2.0 g of 60-200 mesh). Evaporation gave a dry dispersion of crude product in silica gel which was applied atop a column of silica gel. The column was eluted with $CHCl_3$-MeOH (5 : 1), and fractions were examined by TLC. Appropriate fractions were combined and evaporated, and the residue was stirred with Et_2O, then collected by filtration. The yield of diethyl ester (II-6) was 36% (120 mg); mass spectrum, m/e 501, MH+ for $C_{23}H_{28}N_6O_5S$.

N-(5-(2-(2,4-Diamino[2,3-d]pyrimin-6-yl)thiophene-2-carbonyl]-L-glutamic Acid (**II-7**). The ester (**II-6**, 120 mg) was dissolved in MeOH (10 mL) along with 1N NaOH (0.5 mL). After 2 days at 20-23°C in a stoppered flask protected from light, the solution was evaporated in vacuo, bath 25°C, to remove MeOH which was replaced with H_2O (10 mL). More 1N NaOH (0.25 mL) was added, and the aqueous solution was left at 20-23°C for 2 days before it was treated with 1N HCl (pH 4.0). The precipitated product was collected, washed with H_2O, and dried (in vacuo) to afford II-7 in 37% yield (43 mg); MS, m/e 445, MH+. Anal. Calcd for $C_{19}H_{20}N_6O_5S•2H_2O$: C, 47.49; H, 5.03; N, 17.49. Found: C, 47.23; H, 4.81; N, 17.13.

Example G': Synthesis Of Compound 93, Table II, By Procedure IV

3-Carboxypyridine-6-acetic Acid Dimethyl Ester (**III-2**). This diester was similarly prepared as for **II-2** from 6-methylnicotinic acid (**III-1**) as a yellow solid, mp 56-57°C; NMR ($CDCl_3$) d 9.10 (m, 1H, 6-H); 8.21 (m, 1H, 4-H); 7.33 (m, 1H, 3-H); 3.84 (m, 8H, CH_2COOCH_3dArCOOCH$_3$); Anal. ($C_{10}H_{11}NO_4$); Calc: C, 57.4; H, 5.30; N, 6.70. Found: C, 57.5; H, 5.33; N, 6.54.

10-Carbomethoxy-4-deoxy-4-amino-5,10-dideaza-3'-azapteroic Acid Methyl Ester (**III-3**). Alkylation of the sodio derivative of 3-carbomethoxy-6-pyridylacetic acid methyl ester (**III-2**) with **I-3** to produce **III-3** was done as described for the preparation of **II-3**. The product obtained in greater than 90% yield was nearly homogeneous by TLC ($CHCl_3$-MeOH, 5:1) and produced the expected mass spectral peak of m/e 393, MH+ for $C_{18}H_{18}N_6O_4$. Purity assay by HPLC showed the main component to be >86% with respect to UV-absorbing material. This material was used as such for conversion to **III-4**.

10-Carboxy-4-deoxy-4-amino-5,10-dideaza-3'-azapteroic Acid (**III-4**). Ester hydrolysis of III-3 was conducted as described for the conversion of **II-3** to **II-4**. The overall yield of **II-4** from **I-3** was 86% (1.25 g from 1.66 g, 4.08 mmol of I-3). Mass spectrum, m/e 355, MH+ for $C_{16}H_{14}N_6O_4$.

4-Deoxy-4-amino-5,10-dideaza-3'-azapteroic Acid (**III-5**). Compound **III-4** (1.25 g) was suspended in DMF (30 mL), and the stirred mixture was heated at 75°C for 20 min. Evolution of CO_2 appeared to cease after 10 min although solution did not occur. DMF was removed in vacuo, and the residue was stirred with H_2O, collected and dried to give **III-5** (0.88 g, ~84% yield); mass spectrum, m/e 311, MH+ for $C_{15}H_{14}N_6O_2$.

N-[2-[2-[(2,4-Diaminopyrido[2,3-d]pyrimidin-6-yl)ethyl]-5-pyridyl]carbonyl]-L-glutamic Acid (**III-7**). (5,10-Dideaza-3'-azaaminopterin). Compound **III-5** was coupled with diethyl L-glutamate in the same manner as described for compound **II-6** (Example F'). The yield of pure ester, homogeneous by TLC ($CHCl_3$-MeOH, 5 : 1; Rf ~0.5), was about 18% (215 mg from 740 mg, 2.38 mmol of **III-5**); Mass spectrum, m/e 496, MH+ for $C_{24}H_{29}N_7O_5$. Hydrolysis of the ester groups of this product (175 mg, 0.353 mmol) as described under the preparation of **II-7** led to pure **III-7**•$3H_2O$ in 77% yield (119 mg), Mass spectrum, m/e 440; 1H NMR d 1.95, 2.08 (two m, 2, $CHCH_2$ nonequivalent), 2.34 (t, 2, CH_2CO), 3.06 (m, 2, C_9H_2 or $C_{10}H_2$), 3.17 (m, 2, C_9H_2 or $C_{10}H_2$), 4.38 (m, 1, $CHCH_2$), 6.85 (s, 2, NH_2), 7.36 (d, 1, pyridyl-3-H), 7.84 (s, 2, NH_2), 8.12 (m, 1, pyridyl-4-H), 8.37 (d, I, C5-H), 8.54 (d, 1, C_7-H), 8.68 (d, 1, CONH), 8.97 (d, 1, pyridyl-6-H between N and carboxamide). Anal. Calcd for $C_{20}H_{21}N_7O_5$•$3H_2O$: C, 48.68; H, 5.51; N, 19.87. Found: C, 48.45; H, 5.55; N, 19.75.

Example H': Synthesis Of Compound 99, Table IIB, By Procedure IV

10-Carbomethoxy-4-deoxy-4-amino-5-methyl-5,10-dideaza-3'-azapteroic Acid Methyl Ester (**III-8**). NaH (480 mg of 60% in oil, 12.0 mmol) was suspended in DMF (12 mL), and the mixture was chilled to 0°C, then treated with a solution of 3-carbomethoxy-6-pyridylacetic acid methyl ester (**III-2**, 2.50 g, 12.0 mmol) in DMF (12 mL). After 0.5 h at 0°C, the stirred mixture was chilled to -25°C, treated with a solution of I-4•1.7HBr•0.5AcOH (1.71 g, 3.92 mmol) in DMF (12 mL), then allowed to warm to -10°C. After 1 h at near -10°C, the solution was allowed to warm to ambient temperature. After 1 h at 20-23°C, the mixture was

neutralized by the addition of small pieces of solid CO_2. Addition of silica gel (7.5 g of 60-200 mesh) followed, and the resulting mixture was evaporated to dryness (<1 mm, bath to 40°C) to give a dispersion of crude product in silica gel which was applied to a column of silica gel. Elution by $CHCl_3$-MeOH (9 : 1) led to fractions homogeneous in **III-8** according to TLC ($CHCl_3$-MeOH, 3:1; Rf ~0.6). Evaporation of the combined fractions gave **III-8** in 43% yield (668 mg); mass spectrum, m/e 397, MH+ for $C_{19}H_{20}N_6O_4$.

10-Carboxy-4-deoxy-4-amino-5-methyl-5,10-dideaza-3'-azapteroic Acid (**III-9**). A suspension of **III-8** (668 mg, 1.69 mmol) in DMSO was treated with 4N NaOH (1.0 mL). The resulting clear solution was kept under N_2 in a stoppered flask protected from light for 20 h. After the solvent had been removed by short-path distillation in vacuo (<1 mm, bath to 40°C) the residue was dissolved in H_2O (30 mL), and the filtered solution was acidified to pH 5 using glacial AcOH. The mixture was kept several hours in a refrigerator before the solid was collected, washed with H_2O, and dried in vacuo (over P_2O_5). This material was found through HPLC assay results and mass spectral data to be a mixture consisting of 88% compound **III-9** (m/e 369, MH+ for $C_{17}H_{16}N_6O_4$) and 12% of the sequential product **III-10** (m/e 325, MH+ for $C_{16}H_{16}N_6O_2$). The weight of the mixture (594 mg) corresponds to conversion of 97%.

4-Amino-4-deoxy-5-methyl-5,10-dideaza-3'-azapteroic Acid (**III-10**). The above material **III-9** (594 mg) was suspended in DMF (15 mL), and the stirred mixture was kept at 60-65°C for 12 min. Decarboxylation was slow at this temperature, and the bath was raised to 80-85°C. Heating was continued 15 min longer. To ensure complete reaction, DMF was removed in vacuo and replaced with DMSO. The resulting clear solution was kept at 60-65°C for 15 min, then examined by HPLC showing complete decarboxylation. DMSO was removed in vacuo, and the product was precipitated from a clarified (Norit, Celite) aqueous solution of its Na salt by addition of AcOH to pH 5; yield 77% (443 mg); Mass spectrum, m/e 325, MH+. Anal. Calcd for $C_{16}H_{16}N_6O_2$: C, 54.69; H, 5.45; N, 23.92. Found: C, 54.77; H, 5.13; N, 24.41.

N-[2-[2-[(2,4-Diamino-5-methylpyrido[2,3-d]pyrimidin-6-yl)ethyl]-5-pyridyl]carbonyl]-L-glutamic Acid Diethyl Ester (**III-11**). A stirred mixture of **III-10** (382 mg, 1.09 mmol) in DMF (40 mL) was treated with Et_3N (0.61 mL, 0.44 mg, 44 mmol) followed by i-BuOCOCl (0.14 mL, 0.15 g, 1.08 mmol), then stirred at 20-23°C for 15 min before diethyl L-glutamate•HCl (261 mg, 1.09 mmol) was added. Three more additions of i-BuOCOCl (0.55, 0.27, and 0.27 mmol) were made at intervals of 15 min, and each was followed 1 min later by an equimolar amount of diethyl L-glutamate•HCl. The course of the conversion was followed by TLC ($CHCl_3$-MeOH, 3:1), and a chromatogram observed 2 h after the final addition revealed one major UV-absorbing spot. DMF was then removed (<1 mm, 25-30°C). The residue was dissolved in MeOH, and the solution was treated with silica gel (2.0 g of 60-200 mesh). Evaporation gave a dry dispersion of crude product in silica gel which was applied atop a column of silica gel (~300 mL of 230-400 mesh). The column was eluted with $CHCl_3$-MeOH (5:1), and fractions were examined by TLC. Appropriate fractions were combined and

evaporated, and the residue was stirred with Et_2O, then collected by filtration. The air-dried sample was stirred with H2O, and the H2O-insoluble solid was dried to give the diethyl ester (III-11) (281 mg, 47% as dihydrate). Mass spectrum, m/e 510, MH+. Anal. Calcd for $C_{25}H_{31}N_7O_5 \cdot 2H_2O$: C, 55.04; H, 6.47; N, 17.97. Found: C, 55.01; H, 6.23; N, 17.88.

N-[2-[2-[(2,4-Diamino-5-methylpyrido[2,3-d]pyrimidin-6-yl)ethyl]-5-pyridyl]carbonyl[-L-glutamic Acid (III-12). (5-Methyl-5,10-dideaza-3'-azaaminopterin). The ester (261 mg, 0.478 mmol) was dissolved in MeOH (20 mL) containing 1N NaOH (1.1 mL). After 2 days at 20-23°C in a stoppered flask protected from light, the solution was evaporated in vacuo, bath 25°C to remove MeOH which was replaced with H_2O (20 mL). More 1N NaOH (0.5 mL) was added, and the aqueous solution was left at 20-23°C for 2 days longer before it was treated with 1N HCl to pH 4.0 to cause precipitation. The product was collected, washed with H_2O, and dried to give **III-12** in 88% yield (204 mg), Mass spectrum, m/e 454, MH+; UV (lmax, e ¥ 10-3) 0.1N HCl, 228 (38.6), 270 (17.6), 319 (7.96); pH 7, 233 (38.5), 268 (16.6), 334 (6.01); 0.1N NaOH, 233 (37.4), 269 (18.1), 345 (6.72); 1H NMR (Me_2SO-d6) d 1.95, 2.06 (two m, 2, $CHCH_2$ nonequivalent), 2.34 (t, 2, CH_2CO), 2.68 (s, 3, CH_3), 3.06 (m, 4, $C_9H_2C_{10}H_2$), 4.38 (q, 1, $CHCH_2$), 6.90 (s, 2, NH_2), 7.34 (m, 3, NH_2 overlapping pyridyl-3-H), 8.10 (m, 1, pyridyl-4-H), 8.30 (s, 1, C_7-H), 8.64 (d, 1, CONH), 8.96 (d, 1, pyridyl-6-H; between pyridyl N and carboxamide). Anal. Calcd for $C_{21}H_{28}N_7O_5 \cdot 1.6H_2O$: C, 52.30; H, 5.48; N, 20.33. Found: C, 52.19; H, 5.34; N, 20.45.

The deazaaminopterin compound can be administered per se, or in association with a pharmaceutically acceptable diluent or carrier. The invention accordingly also provides a pharmaceutical composition in dosage unit form comprising from 0.1 to about 500 mg of the deazaaminopterin compound, per dosage unit, together with a pharmaceutically acceptable nontoxic inert carrier or diluent therefore.

The deazaaminopterin compound can be used as such, or in the form of an acid addition salt. These salts are formed with one or more free NH_2 groups of the deazaaminopterin molecule. Typically, the compounds are injected in the form of their sodium salts in aqueous solution. Other salts, e.g., K, Ca, NH_4, etc. could be used as prepared from the appropriate hydroxide or carbonates.

The acid addition salts are preferably the pharmaceutically acceptable, nontoxic addition salts with suitable acids, such as those with inorganic acids, for example, hydrochloric, hydrobromic, nitric, sulphuric, and phosphoric acids; and with organic acids, such as organic carboxylic acids, for example, glycolic, maleic, hydroxymaleic, malic, tartaric, citric, calicylic, o-acetyloxybenzoic, nicotinic, and isonicotinic acid; and organic sulphonic acids, for example, methanesulphonic, ethanesulphonic, 2-hydroxyethanesulphonic, toluene-p-sulphonic, and naphthalene-2-sulphonic acid.

An acid addition salt can be converted into the free compound according to known methods, for example, by treating it with a base, such as with a metal hydroxide or alkoxide, for example, an alkali metal or alkaline earth metal hydroxide, for example, lithium hydroxide, sodium hydroxide, potassium hydroxide or calcium hydroxide; with a metal carbonate, such as an alkali metal or an alkaline earth metal carbonate or hydrogen carbonate, for example, sodium, potassium or calcium carbonate or hydrogen carbonate, with ammonia; or with a hydroxyl ion exchange resin, or with any other suitable reagent.

An acid addition salt may also be converted into another acid addition salt according to known methods; for example, a salt with an inorganic acid may be treated with a metal salt, for example a sodium, barium or silver salt, of an acid in a suitable diluent, in which a resulting inorganic salt is insoluble and is thus removed from the reaction medium. An acid-addition salt may also be converted into another acid addition salt by treatment with an anion exchange preparation.

The glutamic acid COOH groups can also be in salt form, as the ammonium NH_4, alkali metal salts (Na^{++}, K^{+}), or the nontoxic alkaline earth metal salts (Ca^{++}) of the glutamate COOH groups.

The deazaaminopterin compound or salt thereof can be administered to the animal by any available route, including oral and parenteral (intravenous, intraperitoneal, subcutaneous, and intramuscular) administration. The amount administered is sufficient to ameliorate the arthritis or other proliferative disease, and will depend upon the type of arthritis, the species of animal, and the weight of the animal. For example, in human administration, a dosage of deazaaminopterin compound within the range from about 0.1 mg/kg to about 500 mg/kg per day should be sufficient. Dosages in the higher part of the range, approaching 500 mg/kg, are normally administered in conjunction with leucovorin (dl-r-formyl tetrahydrofolate) to reduce toxicity. In the treatment of lower test animals, a similar dosage range is therapeutic. The upper limit of dosage is that imposed by toxic side effects, and can be determined by trial and error for the animal to be treated, including humans.

To facilitate administration, the deazaaminopterin compound or salt thereof can be provided in composition form, and preferably in dosage unit form. While the compound can be administered per se, it is normally administered in conjunction with a pharmaceutically acceptable carrier therefor, which dilutes the compound and facilitates handling. The term "pharmaceutically acceptable" means that the carrier (as well as the resulting composition) is sterile and nontoxic.

The carrier or diluent can be solid, semisolid, or liquid, and can serve as a vehicle, excipient, or medium for the compound. Exemplary diluents and carriers include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, alginates, tragacanth, gelatin, syrup, methyl cellulose, polyoxyethylene sorbitan monolaurate, methyl- and propylhydroxybenzoate, talc, or magnesium stearate.

For convenience in handling, the deazaaminopterin compound and carrier or diluent can be enclosed or encapsulated in a capsule, sachet, cachet, gelatin, paper, or other container, especially when intended for use in doage units. The dosage units can for example take the form of tablets, capsules, suppositories, or cachets.

The following Examples 1-7 illustrate various forms of dosage units in which the deazaaminopterin compounds or salts thereof can be prepared:

Example 1

Tablet Formation	Mg/tablet
Deazaaminopterin compound	15
Lactose	86
Corn starch (dried)	45.5
Gelatin	2.5
Magnesium stearate	1.0

The deazaaminopterin compound is powdered and passed through a mesh sieve and well mixed with the lactose and 30 mg of the corn starch, both passed through a sieve.

The mixed powders are massed with a warm gelatin solution, prepared by stirring the gelatin in water and heating to form a 10% w/w solution. The mass is granulated by passing through a sieve, and the moist granules dried at 40°C.

The dried granules are regranulated by passing through a seive and the balance of the starch and the magnesium stearate is added and thoroughly mixed.

The granules are compressed to produce tablets each weighing 150 mg.

Example 2

Tablet Formation	Mg/tablet
Deazaaminopterin compound	100
Lactose	39
Corn starch (dried)	80
Gelatin	4.0
Magnesium stearate	2.0

The method of preparation is identical with that of Example 1, except that 60 mg of starch is used in the granulation process and 20 mg during tableting.

Example 3

Capsule formation	Mg/capsule
Deazaaminopterin compound	250
Lactose	150

The deazaaminopterin compound and lactose are passed through a sieve and the powders well mixed together before filling into hard gelatin capsules of suitable size, so that each capsule contains 400 mg of mixed powders.

Example 4

Suppositories	Mg/suppositories
Deazaaminopterin compound	50
Oil of Theobroma	950

The deazaaminopterin compound is powdered and passed through a sieve and triturated with molten oil of theobroma at 45°C to form a smooth suspension.

The mixture is well stirred and poured into molds, each of nominal 1 g capacity, to product suppositories.

Example 5

Cachets	Mg/cachet
Deazaaminopterin compound	100
Lactose	400

The deazaaminopterin compound is passed through a mesh sieve, mixed with lactose previously sieved and fitted into cachets of suitable size so that each contains 500 mg.

Example 6

Intramuscular injection (sterile suspension in aqueous vehicle)	Mg
Deazaaminopterin compound	10
Sodium citrate	5.7
Sodium carboxymethylcellulose (low viscosity grade)	2.0
Methyl para-hydroxybenzoate	1.5
Propyl para-hydroxylbenzoate	0.2
Water for injection to 1.0 ml	

Example 7

Intraperitoneal intravenous or subcutaneous injection (sterile solution in aqueous carrier system)	Mg
Deazaaminopterin compound, hydrochloric acid addition salt	15
Sodium citrate	5.7
Sodium carboxymethylcellulose (low viscosity grade)	2.0
Methyl para-hydroxybenzoate	1.5
Propyl para-hydroxylbenzoate	0.2
Water for injection to 1.0 ml	

Example 8: In Vivo Biology Of Type II Collagen Arthritis And Methotrexate Treatment Using 5-Deazaaminopterin Compounds In Table 1A Of Formula II Where A Is N

The following data illustrate administration to mice of several deazaaminopterin compounds of the invention and methotrexate in the evaluation of anti-inflammatory activity. The data are presented as two separate observations, the visually observed presence of inflammation in the mouse, and the caliper-measured degree of swelling of the rear paws of the mouse.

The efficacy evaluation used a mouse model of inflammatory disease that occurs in response to an antigenic challenge with Type II collagen [J. S. Courtenay et al., Nature, 283, 666-668 (1980)].

The fundamental aspects of the model allow it to serve as a representative presentation of human disease. The parallels between the known aspects of the mouse model and rheumatoid arthritis include a humoral response in which antibodies are produced to an antigen that is

present in the joint tissue and the antigenic challenge is accompanied by cell-mediated aspects of immunity. The resultant inflammation of the joint tissue yields facets of periostitis, synovial lining hyperplasia, degradation of bone and cartilage and pannus and new bone formation.

The basic elements of the model included the immunization of DBA/1 mice with a suspension of fetal bovine Type II collagen (1 mg/ml) prepared in complete Freund's adjuvant. The primary injection was given using 0.1 ml of the collagen emulsion giving a total of 0.1 mg of Type II collagen per mouse. The animals were then given a booster injection of Type II collagen (100 μg in 0.01 M acetic acid) on day 21 by intraperitoneal injection.

The results of the in vivo testing of methotrexate showed that using prophylactic regimens in which drug was begun two days prior to administration of antigen (Type II collagen) was more effective than starting drug at day 19, two days prior to the first and only boost with Type II collagen. Typically, in this model the untreated positive control animals have an incidence of arthritis ranging from 90 to 100% of injected animals at day 44.

The effect of methotrexate and test compounds on the extent of inflammation was determined by direct analysis of paw swelling using caliper measurements. The results are presented in Table IIA, and show a direct correlation between the decrease in the number of animals having disease and a decrease in the extent of inflammation, as determined by paw swelling.

Table IIA

Compound	Dose (mg/kg)	No Mice on day indicated[b] Day 30	Day 37	Day 44	Ave. thickness of rear paws (mm) over days 30-44[c] Treated	Untreated
None		31/43	38/43	41/43		
5-Me-10-H (5-Me-5-DA)	1.5	0.8	2/8	6/8	(2.18-2.55)	(2.33-2.87)
5-Me-10-Me (5-Me-5-DMTX)	1.5	1/8	4/8	5/8	(2.26-2.44)	(2.33-2.87)
5-Et-10-H (5-Et-5-DA)	1.0	2/8	5/8	2/8	(2.19-2.24)	(2.56-2.98)
5-Et-10-Me (5-Et-5-DMTX)	0.75	0/7	1/7	1/7	(2.20-2.18)	(2.24-2.63)
5-Pr-10-H (5-Pr-5-DA)	1.5	0/7	0/7	0/7	(2.14-2.15)	(2.24-2.63)
5-H-10-Propargyl (10-Prgl-5-DA)	12.0	3/8	2/8	6/8	(2.22-2.37)	(2.34-2.78)
5-Me-10-Propargyl (5-Me-10-Prgl-5-DA)	1.5	3/8	3/8	2/8	(2.29-2.29)	2.56-2.98)
5-Me-10-Allyl (5-Me-10-Allyl-5-DA)	3.0	0/8	0/8	0/8	(2.12-2.16)	(2.32-2.72)
MTX[a]	9.0	1/22	1/22	6/22	(2.18-2.34)	(2.24-2.98)

a Methotrexate (MTX) and untreated controls are composites from multiple runs.

b Visual evidence of inflammation.

c Values in parentheses are 30 day and 44 day measurements vs. equivalent for untreated controls; decrease in inflammation vs. control is most notable at day 44.

It is apparent from the above results that the number of test mice affected was very considerably descreased by administration of deazaaminopterin compound. The results show that deazaaminopterin compound on a similar dosage level to be at least as effective as methotrexate, and since methotrexate is accepted as effective the deazaaminopterin compound is to be expected to be at least as effective as methotrexte, under similar conditions. The potent anti-arthritic activity of the deazaaminopterin compounds tested is evident from the results.

In addition to an effectiveness at least as great methotrexate at a similar dosage level, the compounds of the invention have an advantage in a lower-toxicity, meaning that dosages higher than methotrexate can be possible. The data in Table IIIA show less cytotoxicity on a human liver cell line (Chang liver) than methotrexate, in terms of the rates of cytotoxic potency versus methotrexate, whose ratio in those terms is 1.00. Thus, the higher the ratio is above 1, the lower the toxicity of the compound in respect to methotrexate.

Table IIIA

Ratio Of Cytotoxic Potency Versus Methotrexate For Inhibition Of Human Liver Cell Growth In Culture

Compound	Ratio
MTX	1.00
5-Me-10-NH	4.39
5-Me-10-NMe	2.79
5-Et-10-NH	4.50

Table IIIA

Ratio Of Cytotoxic Potency Versus Methotrexate For Inhibition Of Human Liver Cell Growth In Culture

Compound	Ratio
5-Et-10-NMe	3.18
5-Pr-10-NH	1.78
5-Bu-10-NH	7.20
5-Me-10-N-Propargyl	21.18
5-H-10-N-Propargyl	1.26
5-Me-10-N-allyl	
5,10-Dideazaaminopterin	2.46
10-Me-5,10-Dideazaaminopterin	2.69

Example 9: *In Vivo* Biology Of Type II Collagen Arthritix And Methotrexate Treatment Using 5-Deazaaminopterin Compounds In Table IA Of Formula II Wherein A Is N

The following illustrates administration to mice of several 5-deazaaminopterin compounds of the invention and methotrexate in the evaluation of anti-inflammatory activity. The data are presented in Table IVA below as two separate observations, the visually observed presence of inflammation in the mouse, and the visually observed presence of swelling of the rear paws of the mouse.

The efficacy evaluation uses a mouse model of inflammatory disease that occurs in response to an antigenic challenge with Type II collagen [J. S. Courtenay et al., Nature, 283, 666-668 (1980)], as described in Example 8 hereinabove.

Table IVA

Visual Observation Of Inflammation And Paw Swelling

Compound	Dose (mg/kg)	Visual Observation of Inflammation	Visual Observation of Presence of Swelling
None			
5-Me-10-H 5,10-dideaza	1.5	Yes	Yes
5-Me-10-Me 5,10-dideaza	1.5	Yes	Yes
5-Et-10-H 5,10-dideaza	1.0	Yes	Yes
5-Et-10-Me 5,10-dideaza	0.75	Yes	Yes
5-Pr-10-Me 5,10-dideaza	1.5	Yes	Yes
5-H-10-Propargyl 5,10-dideaza	12.0	Yes	Yes
5-Me-10-Propargyl 5,10-dideaza	1.5	Yes	Yes
5-Me-10-Allyl 5,10-dideaza	3.0	Yes	Yes

The results show the deazaaminopterin compound on a similar dosage level to be at least as effective as methotrexate, and since methotrexate is accepted as effective the deazaaminopterin compound is to be expected to be at least as effective as methotrexate, under similar conditions. The anti-arthritic activity of the deazaaminopterin compounds tested was confirmed by the results.

Example 10: In Vivo Biology Of Type II Collagen Arthritis And Methotrexate Treatment Using Heteroaroyl-5-DeazaaminopterIn Compounds Nos. A' To H' Of Table IB

The following data illustrate administration to mice of Compounds Nos. A' to H' of Table IB of the invention and methotrexate in the evaluation of anti-inflammatory activity. The data are presented as two separate observations, the visually observed presence of inflammation in the mouse, and the caliper-measured degree of swelling of the rear paws of the mouse.

The efficacy evaluation used a mouse model of inflammatory disease that occurs in response to an antigenic challenge with Type II collagen [Courtenay et al., Nature, 283, 666-668 (1980)], as described in Example 8 hereinabove.

The effect of methotrexate and test compounds on the extent of inflammation was determined by direct analysis of paw swelling using caliper measurements. The results are presented in Table IIB, and show a direct correlation between the decrease in the number of animals having disease and a decrease in the extent of inflammation, as determined by paw swelling.

Table IIB

Compound	Dose (mg/kg)	No mice affected on day indicated[b] Day 30	Day 37	Day 44	Avg. thickness of rear paws (mm) over days 30-44[c] Treated	Untreated
None		31/43	38/43	41/43		2.29-2.73
A'						
B'	6.0	0/5	2/5	2/5	2.19-2.33	
C'	6.0	0/8	1/8	3/8	2.13-2.27	
D'	6.0	2/8	4/8	7/8	2.13-2.32	
E'						
F'	8.0	2/8	7/8	7/8	2.19-2.63	
G'	12.0	5/8	7/8	7/8	2.28-2.67	
H'						
MTX	9.0	1/22	1/22	6/22	2.128-2.34	

a MTX and untreated controls are composites from multiple runs.

b Visual evidence of inflammation.

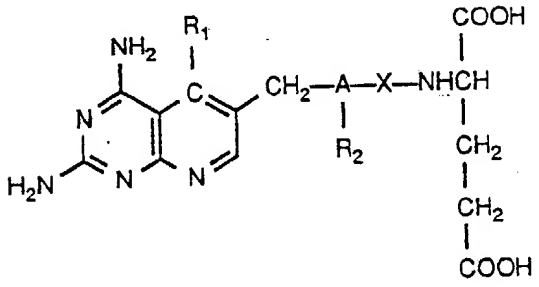
c Values in parentheses are 30 day and 44 day measurements vs. equivalent for untreated controls; decrease in inflammation vs. control is most notable at day 44.

It is apparent from the above results that the number of test mice affected was very considerably decreased by administration of heteroaroyl-5-deazaaminopterin or 5,10-dideazaaminopterin compound. The results show that heteroaroyl-5-deazaaminopterin or 5,10-dideazaaminopterin compound on a similar dosage level to be at least as effective as methotrexate, and since methotrexate is accepted as effective the heteroaroyl-5-deazaaminopterin or 5,10-dideazaaminopterin compound is to be expected to be at least as effective as methotrexate, under similar conditions. The potent anti-arthritic activity of the

heteroaroyl-5-deazaaminopterin or 5,10-dideazaaminopterin compounds tested is evident from the results.

WHAT IS CLAIMED IS:

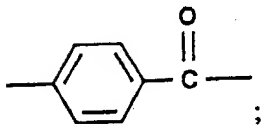
1. 5-deazaaminopterin and 5,10-dideazaaminopterin compounds represented by the formula:



wherein

A is CH or N;

X is one of



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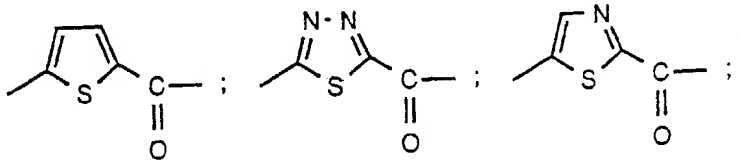
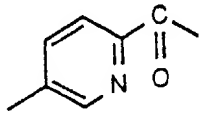


and

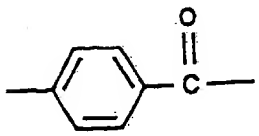


;

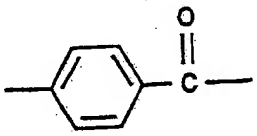
and R_1 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms;

provided that when X is

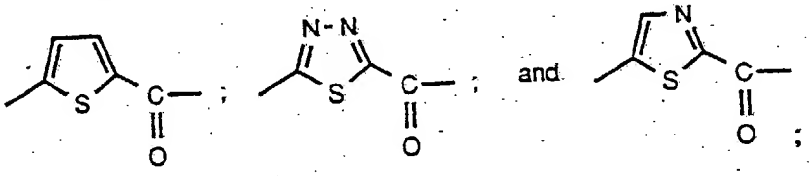


and A is N, then R_1 is alkyl, alkenyl, or alkynyl and when R_1 is alkyl, then R_2 is hydrogen or alkenyl, and further provided that when X is



and A is CH and R_1 is hydrogen or alkyl, then R_2 is alkenyl or alkynl.

2. The compounds of Claim 1 wherein X is one of

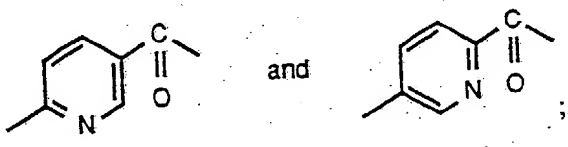


and R_1 is hydrogen or alkyl having from one to about eight carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

3. The compounds of Claim 2 wherein R_1 is hydrogen or alkyl having from one to three carbon atoms, and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to three carbon atoms.

4. The compounds of Claim 1 wherein X is one of

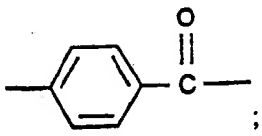


and R_1 is hydrogen or alkyl having from one to about eight carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

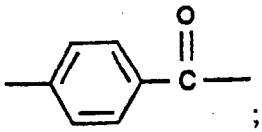
5. The compounds of Claim 4 wherein R_1 is hydrogen or alkyl having from one to about three carbon atoms, and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about three carbon atoms.

6. The compounds of Claim 1 wherein X is



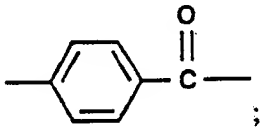
and A is CH, and at least one of R_1 and R_2 is independently alkyl, alkenyl, or alkynyl having from three to about five carbon atoms.

7. The compounds of Claim 1 wherein X is



and A is N, and at least one of R_1 and R_2 is independently alkyl, alkenyl, or alkynyl having from three to about five carbon atoms.

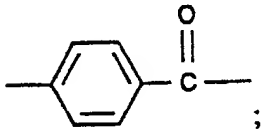
8. The compounds of Claim 7 wherein X is



and at least one of R_1 and R_2 is alkyl having from three to about five carbon atoms.

9. The compounds of Claim 8 wherein the alkyl is propyl or butyl.

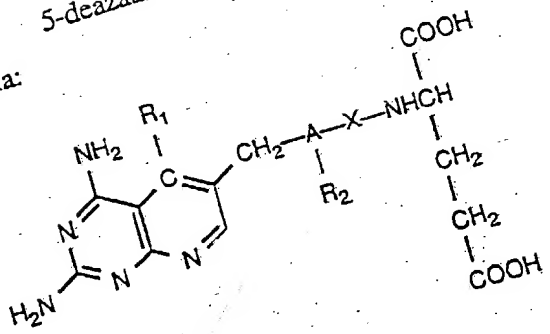
10. The compounds of Claim 1 wherein X is



and at least one of R_1 and R_2 is alkenyl.

11. The compounds of Claim 10 wherein the alkenyl is allyl or butenyl.

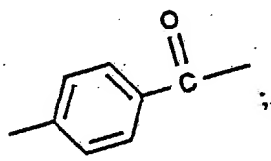
12. 5-deazaaminopterin and 5,10-dideazaaminopterin compounds represented by the formula:

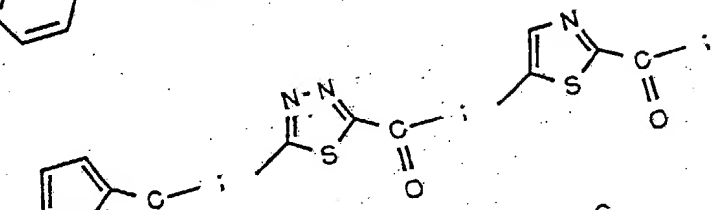


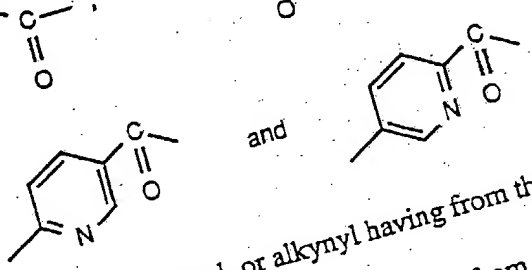
wherein

A is CH or N;

X is one of



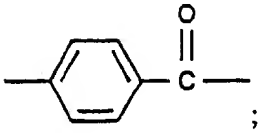




and R_1 is alkyl, alkenyl, or alkynyl having from three to about eight carbon at

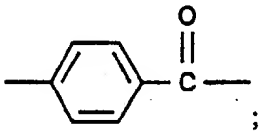
and R_2 is alkyl, alkenyl, or alkynyl having from one to about eight carbon at

13. The compounds of Claim 12 wherein X is



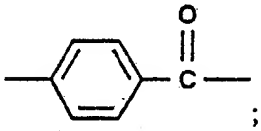
and A is N, and at least one of R_1 and R_2 is independently alkyl or alkenyl having from three to about five carbon atoms.

14. The compounds of Claim 1 wherein X is



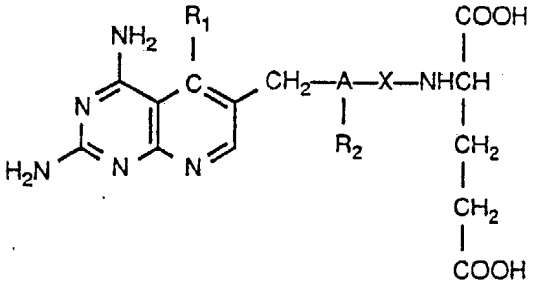
and one of R_1 and R_2 is alkyl and the other of R_1 and R_2 is alkenyl.

15. The compounds of Claim 1 wherein X is



and one of R_1 and R_2 is alkyl and the other of R_1 and R_2 is alkynyl.

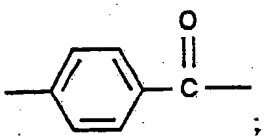
16 A method of treating arthritis and other proliferative diseases which comprises administering to a warm-blooded animal having an inflammation of the joints or other evidence of the diseases, a therapeutic and relatively nontoxic amount of a 5-deazaaminopterin or 5,10-dideazaaminopterin compound having the formula:

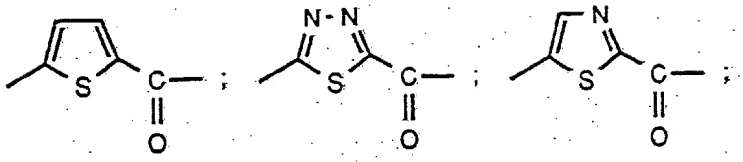


wherein

A is CH or N;

X is one of


;




and

;

and R_1 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

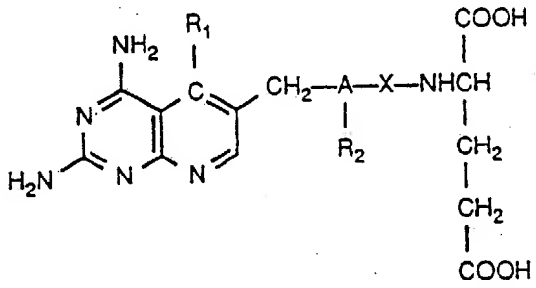
17. The method of Claim 16 wherein the compound is administered as a pharmaceutically acceptable salt thereof.

18. The method of Claim 16 or 17 wherein the compound is administered in an amount within the range from about 0.1 to about 500 mg per day.

19. The method of Claim 16 or 17 wherein the compound is administered with an inert diluent or carrier.

20. The method of Claim 16 or 17 the compound is administered orally or parenterally.

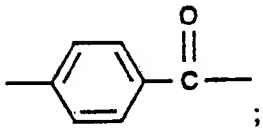
21. A pharmaceutical composition in dosage unit form for treating arthritis or other proliferative disease comprising an amount within the range from about 0.1 to about 500 mg per dosage unit therapeuticaly effective to ameliorate arthritis or other proliferative disease of a 5-deazaaminopterin or 5,10-dideazaaminopterin compound together with a pharmaceutically acceptable nontoxic carrier or diluent thereof; the compound having the formula:



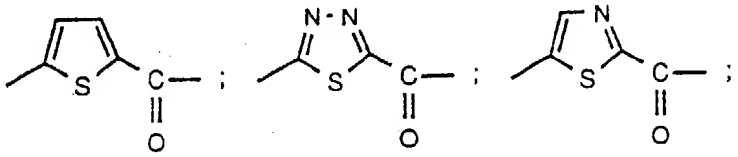
wherein

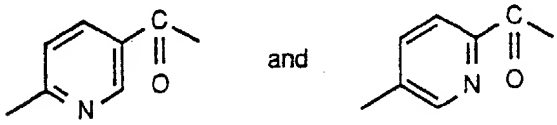
A is CH or N;

X is one of



;





and

;

and R_1 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms;

and R_2 is hydrogen or alkyl, alkenyl, or alkynyl having from one to about eight carbon atoms.

22. The pharmaceutical composition of Claim 13 which is in the form of a pharmaceutically acceptable acid addition salt.

23. The pharmaceutical composition of Claim 21 or 22 in sterile aqueous, aqueous dispersion, capsule, cachet, or suppository form.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03965

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D471/04; A61K31/505; //(C07D471/04,239:00,221:00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 451 835 (MERRELL DOW PHARMACEUTICALS INC.) 16 October 1991 *Document* ---	1-23
A	EP,A,0 451 836 (HEALTH RESEARCH INC.) 16 October 1991 *Document* ---	1-23
A	WO,A,9 000 172 (SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH) 11 January 1990 see page 30 - page 54; claims ---	1-23
A	WO,A,8 605 181 (THE TRUSTEES OF PRINCETON UNIVERSITY) 12 September 1986 see page 37 - page 41; claims ---	1-23

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 AUGUST 1993	-2. 09. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LUYTEN H.W.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	US,A,5 077 404 (JAMES R. PIPER ET. AL.) 31 December 1991 *Document*	1-23
A	US,A,4 725 687 (SOUTHERN RESEARCH INSTITUTE) 16 February 1988 *Document*	1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/ 03965

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 16-20 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9303965
SA 74072

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 19/08/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0451835	16-10-91	US-A-	5066828	19-11-91
		AU-B-	635628	25-03-93
		AU-A-	7416591	17-10-91
		CN-A-	1055740	30-10-91
		JP-A-	4235144	24-08-92
EP-A-0451836	16-10-91	AU-B-	631055	12-11-92
		AU-A-	7417591	17-10-91
		CN-A-	1057653	08-01-92
		JP-A-	4234854	24-08-92
WO-A-9000172	11-01-90	AU-A-	3843189	23-01-90
		EP-A-	0377031	11-07-90
		JP-T-	3500176	17-01-91
WO-A-8605181	12-09-86	AU-B-	578813	03-11-88
		AU-A-	5510886	24-09-86
		CA-A-	1276637	20-11-90
		EP-A-	0215063	25-03-87
		JP-T-	62502535	01-10-87
		SU-A-	1676449	07-09-91
		US-A-	4845216	04-07-89
		US-A-	4927828	22-05-90
		US-A-	5026851	25-06-91
		US-A-	4684653	04-08-87
US-A-5077404	31-12-91	None		
US-A-4725687	16-02-88	None		

EPO FORM P0479